

FORM PTO-1390 (Modified)  
(REV 11-98)

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

## TRANSMITTAL LETTER TO THE UNITED STATES

REDC-1510 USA

DESIGNATED/ELECTED OFFICE (DO/EO/US)

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR

CONCERNING A FILING UNDER 35 U.S.C. 371

09/623533

INTERNATIONAL APPLICATION NO.

INTERNATIONAL FILING DATE

PRIORITY DATE CLAIMED

PCT/US00/13651

17 May 2000 (17.05.00)

17 May 1999 (17.05.99)

TITLE OF INVENTION

LONG LASTING FUSION PEPTIDE INHIBITORS OF VIRAL INFECTION

526 Rec'd PCT/INT 05 SEP 2000

APPLICANT(S) FOR DO/EO/US

BRIDON, Dominique P.; DUFRESNE, Robert S.; BOUDJELLAB, Nissab; ROBITAILLE, Martin; MILNER, Peter G.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☐ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
  - a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau). (Request, Description, Claims, Abstract, Tables, Sequence Listing)
  - b. ☐ has been transmitted by the International Bureau.
  - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☐ A copy of the International Search Report (PCT/ISA/210).
8. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
  - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
  - b. ☐ have been transmitted by the International Bureau.
  - c. ☒ have not been made; however, the time limit for making such amendments has NOT expired.
  - d. ☐ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)). (signed)
11. ☐ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

## Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☒ Certificate of Mailing by Express Mail
20. ☒ Other items or information:

- a. Copy of Sequence Listing in computer readable form as originally filed 17 May 2000 (17.05.00).
- b. Copy of Form PCT/IB/301 -- Notification of Receipt of Record Copy.
- c. Two copies of Form PCT/IB/304 -- Notification Concerning Submission or Transmittal of Priority Document.
- d. Verified Statement (Declaration) Claiming Small Entity Status -- Small Business Concern (signed).

**REDC-1510 USA****CALCULATIONS** PTO USE ONLY

**ENTER APPROPRIATE BASIC FEE AMOUNT =**

**\$840.00**

**\$0.00**

**Multiple Dependent Claims (check if applicable).**

**TOTAL OF ABOVE CALCULATIONS**

**\$1,176.00**

☒

**\$588.00**

**SUBTOTAL =**

**\$588.00**

 $+$ 

**\$0.00**

**TOTAL NATIONAL FEE****\$588.00**☒

**\$40.00**

**TOTAL FEES ENCLOSED**

**\$628.00**

**Amount to be:  
refunded**

\$

**charged**

\$

- ☐ A check in the amount of **\$628.00** to cover the above fees is enclosed.
- ☐ Please charge my Deposit Account No. \_\_\_\_\_ in the amount of \_\_\_\_\_ to cover the above fees.  
A duplicate copy of this sheet is enclosed.
- ☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **12-1420** A duplicate copy of this sheet is enclosed.

**SEND ALL CORRESPONDENCE TO:**

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Michael R. Ward

**SIGNATURE**

MICHAEL R. WARD

NAME \_\_\_\_\_

**38.651**

REGISTRATION NUMBER

**05 September 2000 (05.09.00)**

DATE \_\_\_\_\_

526 Rec'd

05 SEP 2000

09/623533

533 Rec'd PCT/PTO 05 SEP 2000

PATENT

PATENT COOPERATION TREATY  
UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US)

In re Nat'l Phase Application of: )  
 )  
DOMINIQUE P. BRIDON ET AL. )  
 )  
Based Upon International ) CERTIFICATION  
Application No. PCT/US00/13651 ) UNDER  
Int'l Filing Date: 17 May 2000 ) 37 CFR §1.10  
 )  
Serial No.: Not Assigned )  
 )  
A Filing Under 35 U.S.C. 371 on: )  
05 September 2000 (05.09.00) )  
 )  
For: **LONG LASTING FUSION PEPTIDE** )  
**INHIBITORS OF VIRAL INFECTION** )  
 )  
 )  
 )

Assistant Commissioner for Patents  
Box PCT  
Washington, D.C. 20231-0001

Attention: U.S. Designated/Elected Office (DO/EO/US)

Dear Sir:

I hereby certify that this Application for entering the national phase of the above-identified International Application and the documents referred to as enclosed herein are being deposited with the United States Postal Service on this date, **05 September 2000 (05.09.00)**, in an envelope bearing "Express Mail Post Office to Addressee" Mailing Label Number **EL387347602US** addressed to: Assistant Commissioner for Patents, Box PCT, Washington, D.C. 20231-0001, Attention U.S. Designated/Elected Office (DO/EO/US).

Enclosed are:

1. Transmittal Letter to the U.S. Designated/Elected Office (DO/EO/US) Concerning A Filing Under 35 U.S.C. 371, in duplicate;
2. Request, description, claims, abstract, tables, and sequence listing of International Application No. PCT/US00/13651 as originally filed 17 May 2000 (17.05.00);

3. Copy of Sequence Listing in computer readable form as originally filed 17 May 2000 (17.05.00);
4. Form PCT/IB/301 -- Notification of Receipt of Record Copy;
5. Form PCT/IB/304 -- Notification Concerning Submission or Transmittal of Priority Document (two forms);
6. Combined Declaration/Power of Attorney/Claim of Priority **(signed)**;
7. Assignment **(signed)** with Form PTO-1595 (Assignment Recordation Form);
8. Verified Statement (Declaration) Claiming Small Entity Status -- Small Business Concern **(signed)**;
9. Check in the amount of \$628.00; and
10. Acknowledgement Postcard.

Dated: 05 September 2000 (05.09.00)

*Elizabeth A. Reicker*  
Elizabeth A. Reicker

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Attorney Docket No. REDC-1510 USA



Applicant or Patentee: DOMINIQUE P. BRIDON ET AL.

Attorney's

Appln. or Patent No.:

Docket No.: REDC-1510 USAFiled or Issued: HEREWITHFor: LONG LASTING FUSION PEPTIDE INHIBITORS OF VIRAL INFECTION**VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) and 1.27(c)) - SMALL BUSINESS CONCERN**

I hereby declare that I am

☐ the owner of the small business concern identified below:☒ an official of the small business concern empowered to act on behalf of the concern identified below:NAME OF CONCERN CONJUCHEM, INC.ADDRESS OF CONCERN 225 President Kennedy Avenue West, Third Floor, Suite 3950, Montreal, Quebec H2X 3Y8, Canada

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention, entitled LONG LASTING FUSION PEPTIDE INHIBITORS OF VIRAL INFECTION by inventor(s) DOMINIQUE P. BRIDON ET AL. described in

☒ the specification filed herewith with title as listed above.☐ application no. \_\_\_\_\_, filed \_\_\_\_\_.☐ patent no. \_\_\_\_\_, issued \_\_\_\_\_.

If the rights held by the above identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below\* and no rights to the invention are held by any person, other than the inventor, who could not qualify as an independent inventor under 37 CFR 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

\*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27).

NAME \_\_\_\_\_

ADDRESS \_\_\_\_\_

☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

NAME \_\_\_\_\_

ADDRESS \_\_\_\_\_

☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time or paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING Dominique P. BridonTITLE OF PERSON OTHER THAN OWNER Vice President of ResearchADDRESS OF PERSON SIGNING 225 President Kennedy Avenue West, Third Floor, Suite 3950, Montreal, Quebec H2X 3Y8, CanadaSIGNATURE: DATE: Aug 15, 2000

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09/623533

-1- EXPRESS MAIL LABEL NO. EL387347602US  
 Attorney Docket No. REDC-1510 USA

533 Rec'd PCT./PTO 05 SEP 2000

**LONG LASTING FUSION PEPTIDE INHIBITORS OF VIRAL  
 INFECTION**

5

**FIELD OF THE INVENTION**

This invention relates to modified peptides that are inhibitors of viral activity and/or exhibit antifusogenic properties. In particular, this invention relates to modified peptide inhibitors of human immunodeficiency virus (HIV), respiratory syncytial virus (RSV), human parainfluenza virus (HPV), measles virus (MeV), and simian immunodeficiency virus (SIV) with long duration of action for the treatment of the respective viral infections. The invention also relates to conjugates of the modified peptides and endogenous carriers, particularly conjugates of the modified peptides and various mobile blood components, particularly mobile endogenous proteins.

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**BACKGROUND OF THE INVENTION**

Membrane fusion events, while commonplace in normal cell biological processes, are also involved in a variety of disease states, including, for example the entry of enveloped viruses into cells. Peptides are known that inhibit or otherwise disrupt membrane fusion-associated events, including, for example, inhibiting retroviral transmission to uninfected cells. As an example, the synthetic peptides DP-107 and DP-178 derived from separate domains within the human immunodeficiency virus type 1 ("HIV-1") transmembrane ("TM") glycoprotein gp41, are potent inhibitors of HIV-1 infection and HIV induced cell-cell fusion.

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Lambert, et al., "Peptides from Conserved Regions of Paramyxovirus Fusion (F) Proteins are Potent Inhibitors of Viral Fusion," Proc. Natl. Acad. Science U.S.A., March 5, 1996, Vol. 93 (5), pp. 2186-91, discloses that the synthetic peptides DP-107 and DP-178 (T-20), derived from separate domains within the human immunodeficiency virus type 1 (HIV-1) transmembrane (TM) protein, gp41, are potent inhibitors of HIV-1 infection and fusion. Using a

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computer searching strategy (computerized antiviral searching technology, C.A.S.T.) based on the predicted secondary structure of DP-107 and DP-178 (T-20), Lambert, et al. identified conserved heptad repeat domains analogous to the DP-107 and DP-178 regions of HIV-1 gp41 within the glycoproteins of other fusogenic viruses. Antiviral peptides derived from three representative paramyxoviruses, respiratory syncytial virus (RSV), human parainfluenza virus type 3 (HPIV-3), and measles virus (MV) blocked homologous virus-mediated syncytium formation and exhibited  $EC_{50}$  values in the range 0.015-0.250  $\mu$ M. Moreover, these peptides were highly selective for the virus of origin.

U.S. Patent Nos. 6,013,263, 6,017,536 and 6,020,459 incorporated herein in their entirety, likewise disclose that the 36 amino acid peptide DP178 corresponding to amino acids 638 to 673 of gp41 from the HIV-1 isolate LAI (HIV-1<sub>LAI</sub>), and the 38 amino acid peptide DP107 corresponding to amino acids 558-595 of gp41 from the HIV-1<sub>LAI</sub>, both exhibit potent anti-HIV-1 activity.

While many of the anti-viral or anti-fusogenic peptides described in the art exhibit potent anti-viral and/or anti-fusogenic activity, these peptides suffer from short plasma half-lives *in vivo*, primarily due to rapid serum clearance and peptidase and protease activity. This in turn greatly reduces the effective anti-viral activity of the peptides. There is therefore a need for a method of prolonging the half-life of existing anti-viral and/or anti-fusogenic peptides and providing for longer duration of action of these peptides *in vivo*.

## SUMMARY OF THE INVENTION

The present invention meets these and other needs and is directed to modified peptides having anti-viral activity and/or anti-fusogenic activity. These modified peptides provide for an increased stability *in vivo* and a reduced susceptibility to peptidase or protease degradation. These modified peptides thereby minimize, e.g., the need for more frequent, or even continual,

administration of the peptides. The products of varying embodiments of the present invention can be used, e.g., as a prophylactic against and/or treatment for infection of a number of viruses, including human immunodeficiency virus (HIV), human respiratory syncytial virus (RSV), human parainfluenza virus (HPV), measles virus (MeV) and simian immunodeficiency virus (SIV). Modification of other peptides involved in viral transfection (e.g., Hepatitis, Epstein Barr and other related viruses) is also within the scope of the invention.

This invention relates to chemically reactive modifications of peptides exhibiting anti-viral and/or anti-fusogenic activity such that the modified peptides can react with available functionalities on blood components to form stable covalent bonds. In one embodiment of the invention, the modified peptides comprise a reactive group which is reactive with amino groups, hydroxyl groups, or thiol groups on blood components to form stable covalent bonds. In another embodiment of the invention, the reactive group can be a maleimide which is reactive with a thiol group on a blood protein, including a mobile blood protein such as albumin.

In particular, the invention relates to such chemically reactive modifications of DP107 and DP178 peptides and analogs thereof, including peptides comprised of amino acid sequences from other (non-HIV) viruses that correspond to the gp41 region of HIV from which DP107 and DP178 are derived and that exhibit anti-viral or anti-fusogenic activity. More particularly, these peptides can exhibit anti-viral activity against, among others, human respiratory syncytial virus (RSV), human parainfluenza virus (HPV), measles virus (MeV) and simian immunodeficiency virus (SIV). The invention also relates to such chemically reactive modifications of the peptides of SEQ ID NO:1 to SEQ ID NO:86.

The invention also relates to compositions for use in the prevention and/or treatment of viral infection comprising a peptide that exhibits anti-viral activity modified with a reactive group as described. More particularly, the invention relates to such compositions for use in the prevention and/or treatment of AIDS,

human respiratory syncytial virus (RSV), human parainfluenza virus (HPV), measles virus (MeV) and simian immunodeficiency virus (SIV).

### BRIEF DESCRIPTION OF THE TABLES

5           The invention will be better understood by reference to the Tables, in which:

Table 1 lists the commonly occurring amino acids together with their one letter and three letter abbreviations, and common protecting groups.

Table 2 shows DP178 carboxy truncations.

10          Table 3 shows DP178 amino truncations.

Table 4 shows DP107 carboxy truncations.

Table 5 shows DP107 amino truncations.

Table 6 shows HIV-2<sub>NIHZ</sub> DP178 analog carboxy truncations.

Table 7 shows HIV-2<sub>NIHZ</sub> DP178 analog amino truncations.

15          Table 8 shows RSV F2 region DP107 analog carboxy truncations.

Table 9 shows RSV F2 region DP107 analog amino truncations.

Table 10 shows RSV F1 region DP178 analog carboxy truncations.

Table 11 shows RSV F1 region DP178 analog amino truncations.

Table 12 shows HPV3 F1 region DP 178 analog carboxy truncations.

20          Table 13 shows HPV3 F1 region DP 178 analog amino truncations.

Table 14 shows HPV3 F1 region DP107 analog carboxy truncations.

Table 15 shows HPV3 F1 region DP107 analog amino truncations.

Table 16 shows representative anti-RSV peptides.

Table 17 shows representative anti-HPV3 peptides.

25          Table 18 shows representative anti-SIV peptides.

Table 19 shows representative anti-MeV peptides.

### BRIEF DESCRIPTION OF SEQUENCE LISTING

30          The invention will be better understood by reference to the Sequence Listing, in which:

SEQ ID NO:1 shows the peptide sequence of DP178.

SEQ ID NO:2 shows the peptide sequence of DP107

SEQ ID NO:3-9 show peptide sequences of certain DP178 analogs.

5 SEQ ID NO:10-30 show the peptide sequences of RSV F1 region and F2 region corresponding to DP178 and DP107, and representative anti-RSV peptides;

SEQ ID NO:31-62 show the peptide sequences of HPIV3 F1 region corresponding to DP178 and DP107, and representative anti-HPIV3 peptides;

10 SEQ ID NO:63-73 show peptide sequences of SIV corresponding to DP178 and representative anti-SIV peptides; and

SEQ ID NO:74-78 show peptide sequences of MeV corresponding to DP178 and representative anti-MeV peptides.

#### DETAILED DESCRIPTION OF THE INVENTION

15 To ensure a complete understanding of the invention the following definitions are provided:

**Anti-viral peptides:** As used herein, anti-viral peptides shall refer to peptides that inhibit viral infection of cells, by, for example, inhibiting cell-cell  
20 fusion or free virus infection. The route of infection may involve membrane fusion, as occurs in the case of enveloped viruses, or some other fusion event involving viral and cellular structures. Peptides that inhibit viral infection by a particular virus may be referenced with respect to that particular virus, e.g., anti-HIV peptide, anti-RSV peptide, etc.

25 **Antifusogenic peptides:** Antifusogenic peptides are peptides demonstrating an ability to inhibit or reduce the level of membrane fusion events between two or more entities, e.g., virus-cell or cell-cell, relative to the level of membrane fusion that occurs in the absence of the peptide.

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**HIV and anti-HIV peptides:** The human immunodeficiency virus (HIV), which is responsible for acquired immune deficiency syndrome (AIDS), is a member of the lentivirus family of retroviruses. There are two prevalent types of HIV, HIV-1 and HIV-2, with various strain of each having been identified. HIV targets CD-4+ cells, and viral entry depends on binding of the HIV protein gp41 to CD-4+ cell surface receptors. Anti-HIV peptides refer to peptides that exhibit anti-viral activity against HIV, including inhibiting CD-4+ cell infection by free virus and/or inhibiting HIV-induced syncytia formation between infected and uninfected CD-4+ cells.

**SIV and anti-SIV peptides:** Simian immunodeficiency viruses (SIV) are lentiviruses that cause acquired immunodeficiency syndrome (AIDS)-like illnesses in susceptible monkeys. Anti-SIV peptides are peptides that exhibit anti-viral activity against SIV, including inhibiting of infection of cells by the SIV virus and inhibiting syncytia formation between infected and uninfected cells.

**RSV and anti-RSV peptides:** Respiratory syncytial virus (RSV) is a respiratory pathogen, especially dangerous in infants and small children where it can cause bronchiolitis (inflammation of the small air passages) and pneumonia. RSVs are negative sense, single stranded RNA viruses and are members of the *Paramyxoviridae* family of viruses. The route of infection of RSV is typically through the mucous membranes by the respiratory tract, i.e., nose, throat, windpipe and bronchi and bronchioles. Anti-RSV peptides are peptides that exhibit anti-viral activity against RSV, including inhibiting mucous membrane cell infection by free RSV virus and syncytia formation between infection and uninfected cells.

**HPV and anti-HPV peptides:** Human parainfluenza virus (HPIV or HPV), like RSV, is another leading cause of respiratory tract disease, and like RSVs, are negative sense, single stranded RNA viruses that are members of the *Paramyxoviridae* family of viruses. There are four recognized serotypes of

HPIV -- HPIV-1, HPIV-2, HPIV-3 and HPIV-4. HPIV-1 is the leading cause of croup in children, and both HPIV-1 and HPIV-2 cause upper and lower respiratory tract illnesses. HPIV-3 is more often associated with bronchiolitis and pneumonia. Anti-HPV peptides are peptides that exhibit anti-viral activity against HPV, including inhibiting infection by free HPV virus and syncytia formation between infected and uninfected cells.

**MeV and anti-Mev peptides:** Measles virus (VM or MeV) is an enveloped negative, single-stranded RNA virus belonging to the *Paramyxoviridae* family of viruses. Like RSV and HPV, MeV causes respiratory disease, and also produces an immuno-suppression responsible for additional, opportunistic infections. In some cases, MeV can establish infection of the brain leading to severe neurological complications. Anti-MeV peptides are peptides that exhibit anti-viral activity against MeV, including inhibiting infection by free MeV virus and syncytia formation between infected and uninfected cells.

**DP-178 and DP178 analogs:** Unless otherwise indicated explicitly or by context, DP-178 means the 36 amino acid DP-178 peptide corresponding to amino acid residues 638-673 of the gp41 glycoprotein of HIV-1 isolate LAI (HIV<sub>LAI</sub>) and having the sequence:

YTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWF (SEQ ID NO:1)

as well as truncations, deletions and/or insertions thereof. Truncations of the DP178 peptide may comprise peptides of between 3-36 amino acids. Deletions consist of the removal of one or more amino acid residues from the DP178 peptide, and may involve the removal of a single contiguous portion of the peptide sequence or multiple portions. Insertions may comprise single amino acid residues or stretches of residues and may be made at the carboxy or amino terminal end of the DP178 peptide or at a position internal to the peptide.



DP178 peptide analogs are peptides whose amino acid sequences are comprised of the amino acid sequences of peptide regions of viruses other than HIV-1<sub>LAI</sub> that correspond to the gp41 region from which DP178 was derived, as well as an truncations, deletions or insertions thereof. Such other viruses may include, but are not limited to, other HIV isolates such as HIV-2<sub>NIH2</sub>, respiratory syncytial virus (RSV), human parainfluenza virus (HPV), simian immunodeficiency virus (SIV), and measles virus (MeV). DP178 analogs also refer to those peptide sequences identified or recognized by the ALLMOTI5, 107x178x4 and PLZIP search motifs described in U.S. Patent Nos. 6,013,263, 6,017,536 and 6,020,459 and incorporated herein, having structural and/or amino acid motif similarity to DP178. DP178 analogs further refer to peptides described as "DP178-like" as that term is defined in U.S. Patent Nos. 6,013,263, 6,017,536 and 6,020,459.

**DP-107 and DP107 analogs:** Unless otherwise indicated explicitly or by context, DP-107 means the 38 amino acid DP-107 peptide corresponding to amino acid residues 558-595 of the gp41 protein of HIV-1 isolate LAI (HIV<sub>LAI</sub>) and having the sequence:

NNLLRAIEAQQHLLQLTVWQIKQLQARILAVERYLKDQ (SEQ ID NO:2)

as well as truncations, deletions and/or insertions thereof. Truncations of the DP107 peptide may comprise peptides of between 3-38 amino acids. Deletions consist of the removal of one or more amino acid residues from the DP107 peptide, and may involve the removal of a single contiguous portion of the peptide sequence or multiple portions. Insertions may comprise single amino acid residues or stretches of residues and may be made at the carboxy or amino terminal end of the DP107 peptide or at a position internal to the peptide.

DP107 peptide analogs are peptides whose amino acid sequences are comprised of the amino acid sequences of peptide regions of viruses other than HIV-1<sub>LAI</sub> that correspond to the gp41 region from which DP107 was derived, as well as truncations, deletions and/or insertions thereof. Such other viruses may include, but are not limited to, other HIV isolates such as HIV-2<sub>NIH2</sub>, respiratory syncytial virus (RSV), human parainfluenza virus (HPV), simian immunodeficiency virus (SIV), and measles virus (MeV). DP107 analogs also refer to those peptide sequences identified or recognized by the ALLMOT15, 107x178x4 and PLZIP search motifs described in U.S. Patent Nos. 6,013,263, 6,017,536 and 6,020,459 and incorporated herein, having structural and/or amino acid motif similarity to DP107. DP107 analogs further refer to peptides described as "DP107-like" as that term is defined in U.S. Patent Nos. 6,013,263, 6,017,536 and 6,020,459.

**Reactive Groups:** Reactive groups are chemical groups capable of forming a covalent bond. Such reactive groups are coupled or bonded to a DP-107 or DP-178 peptide or analogs thereof or other anti-viral or anti-fusogenic peptide of interest. Reactive groups will generally be stable in an aqueous environment and will usually be carboxy, phosphoryl, or convenient acyl group, either as an ester or a mixed anhydride, or an imidate, thereby capable of forming a covalent bond with functionalities such as an amino group, a hydroxy or a thiol at the target site on mobile blood components. For the most part, the esters will involve phenolic compounds, or be thiol esters, alkyl esters, phosphate esters, or the like.

**Functionalities:** Functionalities are groups on blood components to which reactive groups on modified anti-viral peptides react to form covalent bonds. Functionalities include hydroxyl groups for bonding to ester reactive entities; thiol groups for bonding to maleimides, imidates and thioester groups; amino groups for bonding to carboxy, phosphoryl or acyl groups and carboxyl groups for bonding to amino groups.

**Blood Components:** Blood components may be either fixed or mobile.

Fixed blood components are non-mobile blood components and include tissues, membrane receptors, interstitial proteins, fibrin proteins, collagens, platelets, endothelial cells, epithelial cells and their associated membrane and membranous receptors, somatic body cells, skeletal and smooth muscle cells, neuronal components, osteocytes and osteoclasts and all body tissues especially those associated with the circulatory and lymphatic systems. Mobile blood components are blood components that do not have a fixed situs for any extended period of time, generally not exceeding 5, more usually one minute. These blood components are not membrane-associated and are present in the blood for extended periods of time and are present in a minimum concentration of at least 0.1  $\mu\text{g/ml}$ . Mobile blood components include serum albumin, transferrin, ferritin and immunoglobulins such as IgM and IgG. The half-life of mobile blood components is at least about 12 hours.

**Protective Groups:** Protective groups are chemical moieties utilized to protect peptide derivatives from reacting with themselves. Various protective groups are disclosed herein and in U.S. 5,493,007, which is hereby incorporated by reference. Such protective groups include acetyl, fluorenylmethyloxycarbonyl (Fmoc), t-butyloxycarbonyl (Boc), benzyloxycarbonyl (CBZ), and the like. The specific protected amino acids are depicted in Table 1.

TABLE 1			
NATURAL AMINO ACIDS AND THEIR ABBREVIATIONS			
Name	3-Letter Abbreviation	1-Letter Abbreviation	Modified Amino Acids
Alanine	Ala	A	Fmoc-Ala-OH
Arginine	Arg	R	Fmoc-Arg(Pbf)-OH
Asparagine	Asn	N	Fmoc-Asn(Trt)-OH
Aspartic acid	Asp	D	Asp(tBu)-OH
Cysteine	Cys	C	Fmoc-Cys(Trt)
Glutamic acid	Glu	E	Fmoc-Glu(tBu)-OH
Glutamine	Gln	Q	Fmoc-Gln(Trt)-OH
Glycine	Gly	G	Fmoc-Gly-OH
Histidine	His	H	Fmoc-His(Trt)-OH
Isoleucine	Ile	I	Fmoc-Ile-OH
Leucine	Leu	L	Fmoc-Leu-OH
Lysine	Lys	Z	Boc-Lys(Aloc)-OH
Lysine	Lys	X	Fmoc-Lys(Aloc)-OH
Lysine	Lys	K	Fmoc-Lys(Mtt)-OH
Methionine	Met	M	Fmoc-Met-OH
Phenylalanine	Phe	F	Fmoc-Phe-OH
Proline	Pro	P	Fmoc-Pro-OH
Serine	Ser	S	Fmoc-Ser(tBu)-OH
Threonine	Thr	T	Fmoc-Thr(tBu)-OH
Tryptophan	Trp	W	Fmoc-Trp(Boc)-OH
Tyrosine	Tyr	Y	Boc-Tyr(tBu)-OH
Valine	Val	V	Fmoc-Val-OH

- Linking Groups:** Linking (spacer) groups are chemical moieties that link or connect reactive entities to antiviral or antifusogenic peptides. Linking groups may comprise one or more alkyl moieties, alkoxy moiety, alkenyl moiety, alkynyl moiety or amino moiety substituted by alkyl moieties, cycloalkyl moiety, polycyclic moiety, aryl moiety, polyaryl moieties, substituted aryl moieties, heterocyclic moieties, and substituted heterocyclic moieties. Linking groups may also comprise poly ethoxy amino acids, such as AEA ((2-amino) ethoxy acetic acid) or a preferred linking group AEEA ([2-(2-amino) ethoxy]] ethoxy acetic acid.

5        **Sensitive Functional Groups** – A sensitive functional group is a group of atoms that represents a potential reaction site on an antiviral and/or antifusogenic peptide. If present, a sensitive functional group may be chosen as the attachment point for the linker-reactive group modification. Sensitive functional groups include but are not limited to carboxyl, amino, thiol, and hydroxyl groups.

10        **Modified Peptides** – A modified peptide is an antiviral and/or antifusogenic peptide that has been modified by attaching a reactive group. The reactive group may be attached to the peptide either via a linking group, or optionally without using a linking group. It is also contemplated that one or more additional amino acids may be added to the peptide to facilitate the attachment of the reactive entity. Modified peptides may be administered *in vivo* such that conjugation with blood components occurs *in vivo*, or they may be first conjugated to blood components *in vitro* and the resulting conjugated peptide (as defined below) administered *in vivo*.

15        **Conjugated Peptides** – A conjugated peptide is a modified peptide that has been conjugated to a blood component via a covalent bond formed between the reactive group of the modified peptide and the functionalities of the blood component, with or without a linking group. As used throughout this application, the term “conjugated peptide” can be made more specific to refer to particular conjugated peptides, for example “conjugated DP178” or “conjugated DP107.”

20        Taking into account these definitions, the present invention takes advantage of the properties of existing anti-viral and antifusogenic peptides. The viruses that may be inhibited by the peptides include, but are not limited to all strains of viruses listed, e.g., in U.S. Patent Nos. 6,013,263, 6,017,536 and 6,020,459 at Tables V-VII and IX-XIV therein. These viruses include, e.g., human retroviruses, including HIV-1, HIV-2, and human T-lymphocyte viruses (HTLV-I and HTLV-II), and non-human retroviruses, including bovine leukosis virus, feline sarcoma virus,

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feline leukemia virus, simian immunodeficiency virus (SIV), simian sarcoma virus, simian leukemia, and sheep progress pneumonia virus. Non-retroviral viruses may also be inhibited by the peptides of the present invention, including human respiratory syncytial virus (RSV), canine distemper virus, Newcastle Disease virus, human parainfluenza virus (HPIV), influenza viruses, measles viruses (MeV), Epstein-Barr viruses, hepatitis B viruses, and simian Mason-Pfizer viruses. Non-enveloped viruses may also be inhibited by the peptides of the present invention, and include, but are not limited to, picornaviruses such as polio viruses, hepatitis A virus, enteroviruses, echoviruses, coxsackie viruses, papovaviruses such as papilloma virus, parvoviruses, adenoviruses, and reoviruses.

As an example, the mechanism of action of HIV fusion peptides has been described as discussed in the background section of this application and antiviral and antifusogenic properties of the peptides have been well established. A synthetic peptide corresponding to the carboxyl-terminal ectodomain sequence (for instance, amino acid residues 643-678 of HIV-1 class B, of the LAI strain or residues 638-673 from similar strain as well as residues 558-595) has been shown to inhibit virus-mediated cell-cell fusion completely at low concentration. The fusion peptide competes with the leucine zipper region of the native viral gp41 thus resulting in the interference of the fusion/infection of the virus into the cell.

The focus of the present invention is to modify a selected anti-viral and/or antifusogenic peptide with the DAC (Drug Activity Complex) technology to confer to this peptide improved bio-availability, extended half-life and better distribution through selective conjugation of the peptide onto a protein carrier but without modifying the peptide's anti-viral properties. The carrier of choice (but not limited to) for this invention would be albumin conjugated through its free thiol by an anti-viral and/or antifusogenic peptide modified with a maleimide moiety.

Several peptide sequences have been described in the literature as highly potent for the prevention of HIV-1 fusion/infection. As examples, peptide DP178 binds to a conformation of gp41 that is relevant for fusion. Thus in one embodiment of the invention, DP178 and DP178-like peptides are modified.

Likewise, other embodiments of the invention include modification of DP107 and DP107-like peptide for use against HIV, as well as peptides analagous to DP107 and DP178 that are found in RSV, HPV, MeV and SIV viruses.

5      1.      **DP178 and DP107**

**A.      DP178 Peptides**

          The DP178 peptide corresponds to amino acid residues 638 to 673 of the transmembrane protein gp41 from the HIV-1<sub>LAI</sub> isolate, and has the 36 amino acid  
10      sequence (reading from amino to carboxy terminus):

          NH<sub>2</sub>-YTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWF-COOH (SEQ  
ID NO:1)

15           In addition to the full-length DP178 36-mer, the peptides of this invention include truncations of the DP178 peptide comprising peptides of between 3 and 36 amino acid residues (i.e., peptides ranging in size from a tripeptide to a 36-mer polypeptide), These truncated peptides are shown in Tables 2 and 3.

20           In addition amino acid substitutions of the DP178 peptide are also within the scope of the invention. HIV-1 and HIV-2 enveloped proteins are structurally distinct, but there exists a striking amino acid conservation within the DP178-corresponding regions of HIV-1 and HIV-2. The amino acid conservation is of a periodic nature, suggesting some conservation of structure and/or function.

25           Therefore, one possible class of amino acid substitutions would include those amino acid changes which are predicted to stabilize the structure of the DP178 peptides of the invention. Utilizing the DP178 and DP178 analog sequences described herein, the skilled artisan can readily compile DP178 consensus sequences and ascertain from these, conserved amino acid residues which would  
30      represent preferred amino acid substitutions.

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5 The amino acid substitutions may be of a conserved or non-conserved nature. Conserved amino acid substitutions consist of replacing one or more amino acids of the DP178 peptide sequence with amino acids of similar charge, size, and/or hydrophobicity characteristics, such as, for example, a glutamic acid (E) to aspartic acid (D) amino acid substitution. Non-conserved substitutions consist of replacing one or more amino acids of the DP178 peptide sequence with amino acids possessing dissimilar charge, size, and/or hydrophobicity characteristics, such as, for example, a glutamic acid (E) to valine (V)

10 substitution.

15 Amino acid insertions of DP178 may consist of single amino acid residues or stretches of residues. The insertions may be made at the carboxy or amino terminal end of the DP178 or DP178 truncated peptides, as well as at a position internal to the peptide.

20 Such insertions will generally range from 2 to 15 amino acids in length. It is contemplated that insertions made at either the carboxy or amino terminus of the peptide of interest may be of a broader size range, with about 2 to about 50 amino acids being preferred. One or more such insertions may be introduced into DP178 or DP178 truncations, as long as such insertions result in peptides which may still be recognized by the 107x178x4, ALLMOTI5 or PLZIP search motifs described above.

25 Preferred amino or carboxy terminal insertions are peptides ranging from about 2 to about 50 amino acid residues in length, corresponding to gp41 protein regions either amino to or carboxy to the actual DP178 gp41 amino acid sequence, respectively. Thus, a preferred amino terminal or carboxy terminal amino acid insertion would contain gp41 amino acid sequences found immediately amino to

30 or carboxy to the DP178 region of the gp41 protein.



Deletions of DP178 or DP178 truncations are also within the scope of this invention. Such deletions consist of the removal of one or more amino acids from the DP178 or DP178-like peptide sequence, with the lower limit length of the resulting peptide sequence being 4 to 6 amino acids.

Such deletions may involve a single contiguous or greater than one discrete portion of the peptide sequences. One or more such deletions may be introduced into DP178 or DP178 truncations, as long as such deletions result in peptides which may still be recognized by the 107x178x4, ALLMOTI5 or PLZIP search motifs described above.

**B. DP107 Peptides**

DP107 is a 38 amino acid peptide which exhibits potent antiviral activity, and corresponds to residues 558 to 595 of HIV-1<sub>LAI</sub> isolate transmembrane (TM) gp41 glycoprotein, as shown here:

NH<sub>2</sub>-NNLLRAIEAQQHLLQLTVWQIKQLQARILAVERYLKDQ-COOH  
(SEQ ID NO:2)

In addition to the full-length DP107 38-mer, the DP107 peptides include truncations of the DP107 peptide comprising peptides of between 3 and 38 amino acid residues (i.e., peptides ranging in size from a tripeptide to a 38-mer polypeptide). These peptides are shown in Tables 4 and 5, below.

In addition, amino acid substitutions of the DP178 peptide are also within the scope of the invention. As for DP178, there also exists a striking amino acid conservation within the DP107-corresponding regions of HIV-1 and HIV-2, again of a periodic nature, suggesting conservation of structure and/or function.

Therefore, one possible class of amino acid substitutions includes those amino acid

changes predicted to stabilize the structure of the DP107 peptides of the invention. Utilizing the DP107 and DP107 analog sequences described herein, the skilled artisan can readily compile DP107 consensus sequences and ascertain from these, conserved amino acid residues which would represent preferred amino acid substitutions.

The amino acid substitutions may be of a conserved or non-conserved nature. Conserved amino acid substitutions consist of replacing one or more amino acids of the DP107 peptide sequence with amino acids of similar charge, size, and/or hydrophobicity characteristics, such as, for example, a glutamic acid (E) to aspartic acid (D) amino acid substitution. Non-conserved substitutions consist of replacing one or more amino acids of the DP107 peptide sequence with amino acids possessing dissimilar charge, size, and/or hydrophobicity characteristics, such as, for example, a glutamic acid (E) to valine (V) substitution.

Amino acid insertions may consist of single amino acid residues or stretches of residues. The insertions may be made at the carboxy or amino terminal end of the DP107 or DP107 truncated peptides, as well as at a position internal to the peptide.

Such insertions will generally range from 2 to 15 amino acids in length. It is contemplated that insertions made at either the carboxy or amino terminus of the peptide of interest may be of a broader size range, with about 2 to about 50 amino acids being preferred. One or more such insertions may be introduced into DP107 or DP107 truncations, as long as such insertions result in peptides which may still be recognized by the 107x178x4, ALLMOTI5 or PLZIP search motifs described above.

Preferred amino or carboxy terminal insertions are peptides ranging from about 2 to about 50 amino acid residues in length, corresponding to gp41 protein

regions either amino to or carboxy to the actual DP107 gp41 amino acid sequence, respectively. Thus, a preferred amino terminal or carboxy terminal amino acid insertion would contain gp41 amino acid sequences found immediately amino to or carboxy to the DP107 region of the gp41 protein.

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Deletions of DP107 or DP107 truncations are also within the scope of this invention. Such deletions consist of the removal of one or more amino acids from the DP107 or DP107-like peptide sequence, with the lower limit length of the resulting peptide sequence being 4 to 6 amino acids.

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Such deletions may involve a single contiguous or greater than one discrete portion of the peptide sequences. One or more such deletions may be introduced into DP107 or DP107 truncations, as long as such deletions result in peptides which may still be recognized by the 107x178x4, ALLMOTI5 or PLZIP search motifs.

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DP107 and DP107 truncations are more fully described in U.S. Patent No. 5,656,480, which is incorporated herein by reference in its entirety

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## **2. DP107 and DP178 Analogs**

Peptides corresponding to analogs of the DP178, DP178 truncations, DP107 and DP107 truncation sequences of the invention, described, above, may be found in other viruses, including, for example, non-HIV-1 enveloped viruses, non-

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enveloped viruses and other non-viral organisms.

Such DP178 and DP107 analogs may, for example, correspond to peptide sequences present in transmembrane ("TM") proteins of enveloped viruses and may, correspond to peptide sequences present in non enveloped and nonviral organisms. Such peptides may exhibit antifusogenic activity, antiviral activity, most particularly antiviral activity which is specific to the virus in which their

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native sequences are found, or may exhibit an ability to modulate intracellular processes involving coiled-coil peptide structures.

**A. DP178 analogs**

5 DP178 analogs are peptides whose amino acid sequences are comprised of the amino acid sequences of peptide regions of, for example, other (i.e., other than HIV-1) viruses that correspond to the gp41 peptide region from which DP178 was derived. Such viruses may include, but are not limited to, other HIV-1 isolates and HIV-2 isolates.

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DP178 analogs derived from the corresponding gp41 peptide region of other (i.e., non HIV-1LAI) HIV-1 isolates may include, for example, peptide sequences as shown below.

15 NH<sub>2</sub>-YNTIYTLLEESQNQQEKNEQELLELDKWASLWNWF-COOH (SEQ ID NO:3)

NH<sub>2</sub>-YTGIIYNLLEESQNQQEKNEQELLELDKWANLWNWF-COOH (SEQ ID NO:4)

20 NH<sub>2</sub>-YTSLIYSLLEKSQIQQEKNEQELLELDKWASLWNWF-COOH (SEQ ID NO:5)

The peptides of SEQ ID NO:3, SEQ ID NO:4 and SEQ ID NO:5 are derived from HIV-1<sub>SF2</sub>, HIV-1<sub>RF</sub>, and HIV-1<sub>MN</sub>, respectively. Other DP178 analogs include those derived from HIV-2, including the peptides of SEQ ID NO:6 and  
25 SEQ ID NO:7, which are derived from HIV-2<sub>ROD</sub> and HIV-2<sub>NHZ</sub>, respectively. Still other useful analogs include the peptides of SEQ ID NO:8 and SEQ ID NO:9, which have been demonstrated to exhibit anti-viral activity.

In the present invention, it is preferred that the DP178 analogs represent  
30 peptides whose amino acid sequences correspond to the DP178 region of the gp41

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protein, it is also contemplated that the peptides disclosed herein may, additionally, include amino sequences, ranging from about 2 to about 50 amino acid residues in length, corresponding to gp41 protein regions either amino to or carboxy to the actual DP178 amino acid sequence.

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Table 6 and Table 7 show some possible truncations of the HIV-2<sub>NIH2</sub> DP178 analog, which may comprise peptides of between 3 and 36 amino acid residues (i.e., peptides ranging in size from a tripeptide to a 36-mer polypeptide). Peptide sequences in these tables are listed from amino (left) to carboxy (right) terminus.

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**B. Additional DP178 Analogs and DP107 Analogs**

DP178 and DP107 analogs are recognized or identified, for example, by utilizing one or more of the 107x178x4, ALLMOTI5 or PLZIP computer-assisted search strategies described above. The search strategy identifies additional peptide regions which are predicted to have structural and/or amino acid sequence features similar to those of DP107 and/or DP178.

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The search strategies are described fully in the example presented in Section 9 of US Patent Nos. 6,013,263, 6,017,536 and 6,020,459. While this search strategy is based, in part, on a primary amino acid motif deduced from DP107 and DP178, it is not based solely on searching for primary amino acid sequence homologies, as such protein sequence homologies exist within, but not between major groups of viruses. For example, primary amino acid sequence homology is high within the TM protein of different strains of HIV-1 or within the TM protein of different isolates of simian immunodeficiency virus (SIV).

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The computer search strategy disclosed in US Patent Nos. 6,013,263, 6,017,536 and 6,020,459 successfully identified regions of proteins similar to

DP107 or DP178. This search strategy was designed to be used with a commercially-available sequence database package, preferably PC/Gene.

In US Patent Nos. 6,013,263, 6,017,536 and 6,020,459, a series of search motifs, the 107x178x4, ALLMOTIS and PLZIP motifs, were designed and engineered to range in stringency from strict to broad, with 107x178x4 being preferred. The sequences identified via such search motifs, such as those listed in Tables V-XIV, of US Patent Nos. 6,013,263, 6,017,536 and 6,020,459 and included in this application by incorporation by reference, potentially exhibit antifusogenic, such as antiviral, activity, may additionally be useful in the identification of antifusogenic, such as antiviral, compounds.

### 3. Other Anti-Viral Peptides

#### A. Anti-RSV Peptides

Anti-RSV peptides include DP178 and/or DP107 analogs identified from corresponding peptide sequences in RSV which have further been identified to inhibit viral infection by RSV. Such peptides of interest include the peptides of Table 16 and peptides of SEQ ID NO:10 to SEQ ID NO:30. Of particular interest are the following peptides:

YTSVITIELSNIKENKCNCAKVKLIKQELDKYK (SEQ ID NO:14)  
TSVITIELSNIKENKCNCAKVKLIKQELDKYKN (SEQ ID NO:15)  
VITIELSNIKENKCNCAKVKLIKQELDKYKNAV (SEQ ID NO:16)

IALSTNKAVVSLNGVSVLTSKVLDLKNYIDK (SEQ ID NO:29)

The peptide of SEQ ID NO:10 is derived from the F2 region of RSV and was identified in U.S. Patent Nos. 6,103,236 and 6,020,459 using the search motifs described as corresponding to DP107 and DP178 peptides (i.e., "DP107/178 like").

The peptides of SEQ ID NO:14 to SEQ ID NO:16 each have amino acid sequences contained within the peptide of SEQ ID NO:10 and each has been shown to exhibit anti-RSV activity, in particular, inhibiting fusion and syncytia

formation between RSV-infected and uninfected Hep-2 cells at concentrations of less than 50 µg/ml.

The peptide of SEQ ID NO:11 is derived from the F1 region of RSV and was identified in U.S. Patent Nos. 6,103,236 and 6,020,459 using the search motifs described as corresponding to DP107 (i.e., "DP107-like"). The peptide of SEQ ID NO:29 contains amino acid sequences contained within the peptide of SEQ ID NO:10 and likewise has been shown to exhibit anti-RSV activity, in particular, inhibiting fusion and syncytia formation between RSV-infected and uninfected Hep-2 cells at concentrations of less than 50 µg/ml.

#### **B. Anti-HPIV Peptides**

Anti-HPIV peptides include DP178 and/or DP107 analogs identified from corresponding peptide sequences in HPIV and which have further been identified to inhibit viral infection by HPIV. Such peptides of interest include the peptides of Table 17 and SEQ ID NO:31 to SEQ ID NO:62. Of particular interest are the following peptides:

VEAKQARSDIEKLKEAIRDTNKAVQSVQSSIGNLI (SEQ ID NO:52)  
RSDIEKLKEAIRDTNKAVQSVQSSIGNLIVAIKSV (SEQ ID NO:58)

NSVALDPIDISIELNKA KSDLEESKEWIRRSNQKL (SEQ ID NO:35)  
ALDPIDISIELNKA KSDLEESKEWIRRSNQKLDSI (SEQ ID NO:38)  
LDPIDISIELNKA KSDLEESKEWIRRSNQKLDSIG (SEQ ID NO:39)  
DPIDISIELNKA KSDLEESKEWIRRSNQKLDSIGN (SEQ ID NO:40)  
PIDISIELNKA KSDLEESKEWIRRSNQKLDSIGNW (SEQ ID NO:41)  
IDISIELNKA KSDLEESKEWIRRSNQKLDSIGNWH (SEQ ID NO:42)

The peptide of SEQ ID NO:31 is derived from the F1 region of HPIV-3 and was identified in U.S. Patent Nos. 6,103,236 and 6,020,459 using the search motifs described as corresponding to DP107 (i.e., "DP107-like"). The peptides of SEQ

ID NO:52 and SEQ ID NO:58 each have amino acid sequences contained within the peptide of SEQ ID NO:30 and each has been shown to exhibit anti-HPIV-3 activity, in particular, inhibiting fusion and syncytia formation between HPIV-3-infected Hep2 cells and uninfected CV-1W cells at concentrations of less than 1  $\mu$ g/ml.

The peptide of SEQ ID NO:32 is also derived from the F1 region of HPIV-3 and was identified in U.S. Patent Nos. 6,103,236 and 6,020,459 using the search motifs described as corresponding to DP178 (i.e., "DP178-like"). The peptides of SEQ ID NO:35 and SEQ ID NO:38 to SEQ ID NO:42 each have amino acid sequences contained within the peptide of SEQ ID NO:32 and each also has been shown to exhibit anti-HPIV-3 activity, in particular, inhibiting fusion and syncytia formation between HPIV-3-infected Hep2 cells and uninfected CV-1W cells at concentrations of less than 1  $\mu$ g/ml.

### C. Anti-MeV Peptides

Anti-MeV peptides are DP178 and/or DP107 analogs identified from corresponding peptide sequences in measles virus (MeV) which have further been identified to inhibit viral infection by the measles virus. Such peptides of particular interest include the peptides of Table 19 and peptides of SEQ ID NO:74 to SEQ ID NO:86. Of particular interest are the peptides listed below.

HRIDLGPPISLERLDVGTNLGNIAIAKLEAKELLE (SEQ ID NO:77)  
IDLGPPISLERLDVGTNLGNIAIAKLEAKELLESS (SEQ ID NO:79)  
LGPPISLERLDVGTNLGNIAIAKLEAKELLESSDQ (SEQ ID NO:81)  
PISLERLDVGTNLGNIAIAKLEAKELLESSDQILR (SEQ ID NO:84)

Sequences derived from measles virus were identified in U.S. Patent Nos. 6,103,236 and 6,020,459 using the search motifs described as corresponding to



DP178 (i.e., "DP178-like"). The peptides of SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81 and SEQ ID NO:83 each have amino acid sequences so identified, and each has been shown to exhibit anti-MeV activity, in particular, inhibiting fusion and syncytia formation between MeV-infected Hep2 and uninfected Vero cells at concentrations of less than 1 µg/ml.

#### **D. Anti-SIV Peptides**

Anti-SIV peptides are DP178 and/or DP107 analogs identified from corresponding peptide sequences in SIV which have further been identified to inhibit viral infection by SIV. Such peptides of interest include the peptides of Table 18 and peptides of SEQ ID NO:63 to SEQ ID NO:73. Of particular interest are the following peptides:

WQEWKRVDFLEENITALLEEAIQQEKNMYELQK	(SEQ ID NO:64)
QEWKRVDFLEENITALLEEAIQQEKNMYELQKL	(SEQ ID NO:65)
EWKRVDFLEENITALLEEAIQQEKNMYELQKLN	(SEQ ID NO:66)
WERKRVDFLEENITALLEEAIQQEKNMYELQKLNS	(SEQ ID NO:67)
ERKRVDFLEENITALLEEAIQQEKNMYELQKLNSW	(SEQ ID NO:68)
RKRVDFLEENITALLEEAIQQEKNMYELQKLNSWD	(SEQ ID NO:69)
KRVDFLEENITALLEEAIQQEKNMYELQKLNSWDV	(SEQ ID NO:70)
VDFLEENITALLEEAIQQEKNMYELQKLNSWDVF	(SEQ ID NO:71)
DFLEENITALLEEAIQQEKNMYELQKLNSWDVFG	(SEQ ID NO:72)
FLEENITALLEEAIQQEKNMYELQKLNSWDVFGN	(SEQ ID NO:73)

Sequences derived from SIV transmembrane fusion protein were identified in U.S. Patent Nos. 6,103,236 and 6,020,459 using the search motifs described as corresponding to DP178 (i.e., "DP178-like"). The peptides of SEQ ID NO:64 to SEQ ID NO:73 each have amino acid sequences so identified, and each has been shown to exhibit potent anti-SIV activity as crude peptides.

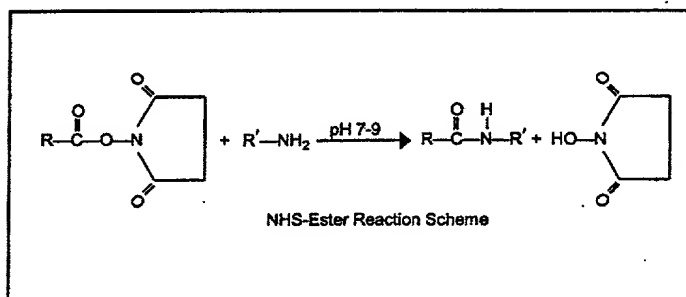
#### **4. Modification of Anti-Viral and Antifusogenic Peptides**

The invention contemplates modifying peptides that exhibit anti-viral and/or antifusogenic activity, including such modifications of DP-107 and DP-178 and analogs thereof. Such modified peptides can react with the available reactive

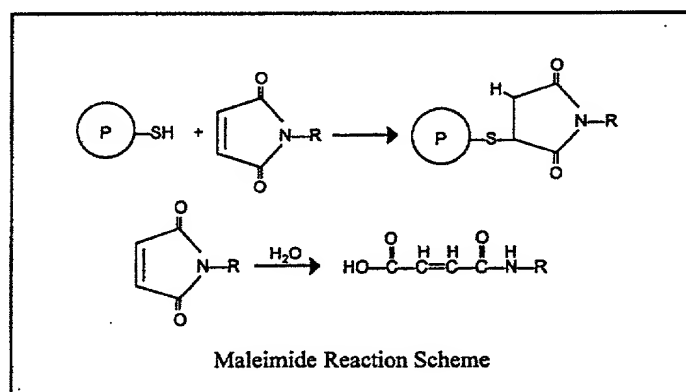
functionalities on blood components via covalent linkages. The invention also relates to such modifications, such combinations with blood components, and methods for their use. These methods include extending the effective therapeutic life of the conjugated anti-viral peptides derivatives as compared to administration of the unconjugated peptides to a patient. The modified peptides are of a type designated as a DAC (Drug Affinity Complex) which comprises the anti-viral peptide molecule and a linking group together with a chemically reactive group capable of reaction with a reactive functionality of a mobile blood protein. By reaction with the blood component or protein the modified peptide, or DAC, may be delivered via the blood to appropriate sites or receptors.

To form covalent bonds with functionalities on the protein, one may use as a reactive group a wide variety of active carboxyl groups, particularly esters, where the hydroxyl moiety is physiologically acceptable at the levels required to modify the peptide. While a number of different hydroxyl groups may be employed in these reactive groups, the most convenient would be N-hydroxysuccinimide or (NHS), N-hydroxy-sulfosuccinimide (sulfo-NHS). In preferred embodiments of this invention, the functionality on the protein will be a thiol group and the reactive group will be a maleimido-containing group such as gamma-maleimide-butyralamide (GMBA) or maleimidopropionic acid (MPA)

Primary amines are the principal targets for NHS esters. Accessible  $\alpha$ -amine groups present on the N-termini of proteins react with NHS esters. However,  $\alpha$ -amino groups on a protein may not be desirable or available for the NHS coupling. While five amino acids have nitrogen in their side chains, only the  $\epsilon$ -amine of lysine reacts significantly with NHS esters. An amide bond is formed when the NHS ester conjugation reaction reacts with primary amines releasing N-hydroxysuccinimide as demonstrated in the schematic below.



In the preferred embodiments of this invention, the functional group on this protein will be a thiol group and the chemically reactive group will be a maleimido-containing group such as MPA or GMBA (gamma-maleimide-butylalamide). The maleimido group is most selective for sulfhydryl groups on peptides when the pH of the reaction mixture is kept between 6.5 and 7.4. At pH 7.0, the rate of reaction of maleimido groups with sulfhydryls is 1000-fold faster than with amines. A stable thioether linkage between the maleimido group and the sulfhydryl is formed which cannot be cleaved under physiological conditions, as demonstrated in the following schematic.



#### A. Specific Labeling.

Preferably, the modified peptides of this invention are designed to specifically react with thiol groups on mobile blood proteins. Such reaction is preferably established by covalent bonding of the peptide modified with a

maleimide link (e.g. prepared from GMBS, MPA or other maleimides) to a thiol group on a mobile blood protein such as serum albumin or IgG.

Under certain circumstances, specific labeling with maleimides offers several advantages over non-specific labeling of mobile proteins with groups such as NHS and sulfo-NHS. Thiol groups are less abundant *in vivo* than amino groups. Therefore, the maleimide-modified peptides of this invention, i.e., maleimide peptides, will covalently bond to fewer proteins. For example, in albumin (the most abundant blood protein) there is only a single thiol group. Thus, peptide-maleimide-albumin conjugates will tend to comprise approximately a 1:1 molar ratio of peptide to albumin. In addition to albumin, IgG molecules (class II) also have free thiols. Since IgG molecules and serum albumin make up the majority of the soluble protein in blood they also make up the majority of the free thiol groups in blood that are available to covalently bond to maleimide-modified peptides.

Further, even among free thiol-containing blood proteins, including IgGs, specific labeling with maleimides leads to the preferential formation of peptide-maleimide-albumin conjugates, due to the unique characteristics of albumin itself.

The single free thiol group of albumin, highly conserved among species, is located at amino acid residue 34 (Cys<sup>34</sup>). It has been demonstrated recently that the Cys<sup>34</sup> of albumin has increased reactivity relative to free thiols on other free thiol-containing proteins. This is due in part to the very low pK value of 5.5 for the Cys<sup>34</sup> of albumin. This is much lower than typical pK values for cysteine residues in general, which are typically about 8. Due to this low pK, under normal physiological conditions Cys<sup>34</sup> of albumin is predominantly in the ionized form, which dramatically increases its reactivity. In addition to the low pK value of Cys<sup>34</sup>, another factor which enhances the reactivity of Cys<sup>34</sup> is its location, which is in a crevice close to the surface of one loop of region V of albumin. This location makes Cys<sup>34</sup> very available to ligands of all kinds, and is an important factor in Cys<sup>34</sup>'s biological role as free radical trap and free thiol scavenger. These properties make Cys<sup>34</sup> highly reactive with maleimide-peptides, and the reaction

rate acceleration can be as much as 1000-fold relative to rates of reaction of maleimide-peptides with other free-thiol containing proteins.

Another advantage of peptide-maleimide-albumin conjugates is the reproducibility associated with the 1:1 loading of peptide to albumin specifically at Cys<sup>34</sup>. Other techniques, such as glutaraldehyde, DCC, EDC and other chemical activations of, e.g, free amines, lack this selectivity. For example, albumin contains 52 lysine residues, 25-30 of which are located on the surface of albumin and therefore accessible for conjugation. Activating these lysine residues, or alternatively modifying peptides to couple through these lysine residues, results in a heterogenous population of conjugates. Even if 1:1 molar ratios of peptide to albumin are employed, the yield will consist of multiple conjugation products, some containing 0, 1, 2 or more peptides per albumin, and each having peptides randomly coupled at any one or more of the 25-30 available lysine sites. Given the numerous possible combinations, characterization of the exact composition and nature of each conjugate batch becomes difficult, and batch-to-batch reproducibility is all but impossible, making such conjugates less desirable as a therapeutic. Additionally, while it would seem that conjugation through lysine residues of albumin would at least have the advantage of delivering more therapeutic agent per albumin molecule, studies have shown that a 1:1 ratio of therapeutic agent to albumin is preferred. In an article by Stehle, et al., "The Loading Rate Determines Tumor Targeting properties of Methotrexate-Albumin Conjugates in Rats," Anti-Cancer Drugs, Vol. 8, pp. 677-685 (1988), incorporated herein in its entirety, the authors report that a 1:1 ratio of the anti-cancer methotrexate to albumin conjugated via glutaraldehyde gave the most promising results. These conjugates were preferentially taken up by tumor cells, whereas conjugates bearing 5:1 to 20:1 methotrexate molecules had altered HPLC profiles and were quickly taken up by the liver *in vivo*. It is postulated that at these higher ratios, conformational changes to albumin diminish its effectiveness as a therapeutic carrier.

Through controlled administration of maleimide-peptides *in vivo*, one can control the specific labeling of albumin and IgG *in vivo*. In typical administrations, 80-90% of the administered maleimide-peptides will label albumin and less than 5% will label IgG. Trace labeling of free thiols such as glutathione will also occur. Such specific labeling is preferred for *in vivo* use as it permits an accurate calculation of the estimated half-life of the administered agent.

In addition to providing controlled specific *in vivo* labeling, maleimide-peptides can provide specific labeling of serum albumin and IgG *ex vivo*. Such *ex vivo* labeling involves the addition of maleimide-peptides to blood, serum or saline solution containing serum albumin and/or IgG. Once conjugation has occurred *ex vivo* with the maleimide-peptides, the blood, serum or saline solution can be readministered to the patient's blood for *in vivo* treatment.

In contrast to NHS-peptides, maleimide-peptides are generally quite stable in the presence of aqueous solutions and in the presence of free amines. Since maleimide-peptides will only react with free thiols, protective groups are generally not necessary to prevent the maleimide-peptides from reacting with itself. In addition, the increased stability of the modified peptide permits the use of further purification steps such as HPLC to prepare highly purified products suitable for *in vivo* use. Lastly, the increased chemical stability provides a product with a longer shelf life.

#### **B. Non-Specific Labeling.**

The anti-viral peptides of the invention may also be modified for non-specific labeling of blood components. Bonds to amino groups will also be employed, particularly with the formation of amide bonds for non-specific labeling. To form such bonds, one may use as a chemically reactive group a wide variety of active carboxyl groups, particularly esters, where the hydroxyl moiety is physiologically acceptable at the levels required. While a number of different hydroxyl groups may be employed in these linking agents, the most convenient would be N-hydroxysuccinimide (NHS) and N-hydroxy-sulfosuccinimide (sulfo-

NHS).

Other linking agents which may be utilized are described in U.S. Patent 5,612,034, which is hereby incorporated herein.

5 The various sites with which the chemically reactive group of the modified peptides may react *in vivo* include cells, particularly red blood cells (erythrocytes) and platelets, and proteins, such as immunoglobulins, including IgG and IgM, serum albumin, ferritin, steroid binding proteins, transferrin, thyroxin binding protein,  $\alpha$ -2-macroglobulin, and the like. Those receptors with which the modified peptides react, which are not long-lived, will generally be eliminated  
10 from the human host within about three days. The proteins indicated above (including the proteins of the cells) will remain at least three days, and may remain five days or more (usually not exceeding 60 days, more usually not exceeding 30 days) particularly as to the half life, based on the concentration in the blood.

For the most part, reaction will be with mobile components in the blood,  
15 particularly blood proteins and cells, more particularly blood proteins and erythrocytes. By "mobile" is intended that the component does not have a fixed situs for any extended period of time, generally not exceeding 5 minutes, more usually one minute, although some of the blood component may be relatively stationary for extended periods of time. Initially, there will be a relatively  
20 heterogeneous population of functionalized proteins and cells. However, for the most part, the population within a few days will vary substantially from the initial population, depending upon the half-life of the functionalized proteins in the blood stream. Therefore, usually within about three days or more, IgG will become the predominant functionalized protein in the blood stream.

25 Usually, by day 5 post-administration, IgG, serum albumin and erythrocytes will be at least about 60 mole %, usually at least about 75 mole %, of the conjugated components in blood, with IgG, IgM (to a substantially lesser extent) and serum albumin being at least about 50 mole %, usually at least about 75 mole %, more usually at least about 80 mole %, of the non-cellular conjugated  
30 components.

The desired conjugates of non-specific modified peptides to blood components may be prepared *in vivo* by administration of the modified peptides to the patient, which may be a human or other mammal. The administration may be done in the form of a bolus or introduced slowly over time by infusion using metered flow or the like.

If desired, the subject conjugates may also be prepared *ex vivo* by combining blood with modified peptides of the present invention, allowing covalent bonding of the modified peptides to reactive functionalities on blood components and then returning or administering the conjugated blood to the host. Moreover, the above may also be accomplished by first purifying an individual blood component or limited number of components, such as red blood cells, immunoglobulins, serum albumin, or the like, and combining the component or components *ex vivo* with the chemically reactive modified peptides. The functionalized blood or blood component may then be returned to the host to provide *in vivo* the subject therapeutically effective conjugates. The blood also may be treated to prevent coagulation during handling *ex vivo*.

## **5. Synthesis of Modified Anti-Viral and Anti-Fusogenic Peptides**

### **A. Peptide Synthesis**

Anti-viral and/or anti-fusogenic peptides according to the present invention may be synthesized by standard methods of solid phase peptide chemistry known to those of ordinary skill in the art. For example, peptides may be synthesized by solid phase chemistry techniques following the procedures described by Steward and Young (Steward, J. M. and Young, J. D., Solid Phase Peptide Synthesis, 2nd Ed., Pierce Chemical Company, Rockford, Ill., (1984) using an Applied Biosystem synthesizer. Similarly, multiple peptide fragments may be synthesized then linked together to form larger peptides. These synthetic peptides can also be made with amino acid substitutions at specific locations.

For solid phase peptide synthesis, a summary of the many techniques may be found in J. M. Stewart and J. D. Young, Solid Phase Peptide Synthesis, W. H.



Freeman Co. (San Francisco), 1963 and J. Meienhofer, Hormonal Proteins and Peptides, vol. 2, p. 46, Academic Press (New York), 1973. For classical solution synthesis see G. Schroder and K. Lupke, The Peptides, Vol. 1, Academic Press (New York). In general, these methods comprise the sequential addition of one or  
5 more amino acids or suitably protected amino acids to a growing peptide chain. Normally, either the amino or carboxyl group of the first amino acid is protected by a suitable protecting group. The protected or derivatized amino acid is then either attached to an inert solid support or utilized in solution by adding the next amino acid in the sequence having the complimentary (amino or carboxyl) group  
10 suitably protected and under conditions suitable for forming the amide linkage. The protecting group is then removed from this newly added amino acid residue and the next amino acid (suitably protected) is added, and so forth.

After all the desired amino acids have been linked in the proper sequence, any remaining protecting groups (and any solid support) are removed sequentially  
15 or concurrently to afford the final polypeptide. By simple modification of this general procedure, it is possible to add more than one amino acid at a time to a growing chain, for example, by coupling (under conditions which do not racemize chiral centers) a protected tripeptide with a properly protected dipeptide to form, after deprotection, a pentapeptide.

20 A particularly preferred method of preparing compounds of the present invention involves solid phase peptide synthesis wherein the amino acid  $\alpha$ -N-terminal is protected by an acid or base sensitive group. Such protecting groups should have the properties of being stable to the conditions of peptide linkage formation while being readily removable without destruction of the growing  
25 peptide chain or racemization of any of the chiral centers contained therein. Suitable protecting groups are 9-fluorenylmethyloxycarbonyl (Fmoc), t-butylloxycarbonyl (Boc), benzyloxycarbonyl (Cbz), biphenylisopropylloxycarbonyl, t-amylloxycarbonyl, isobornylloxycarbonyl,  $\alpha$ ,  $\alpha$ -dimethyl-3,5-dimethoxybenzyloxycarbonyl, o-nitrophenylsulfenyl, 2-cyano-t-butylloxycarbonyl,  
30 and the like. The 9-fluorenyl-methyloxycarbonyl (Fmoc) protecting group is

particularly preferred for the synthesis of the peptides of the present invention. Other preferred side chain protecting groups are, for side chain amino groups like lysine and arginine, 2,2,5,7,8-pentamethylchroman-6-sulfonyl (pmc), nitro, p-toluenesulfonyl, 4-methoxybenzene-sulfonyl, Cbz, Boc, and  
5 adamantyloxycarbonyl; for tyrosine, benzyl, o-bromobenzyloxycarbonyl, 2,6-dichlorobenzyl, isopropyl, t-butyl (t-Bu), cyclohexyl, cyclopropyl and acetyl (Ac); for serine, t-butyl, benzyl and tetrahydropyranyl; for histidine, trityl, benzyl, Cbz, p-toluenesulfonyl and 2,4-dinitrophenyl; for tryptophan, formyl; for aspartic acid and glutamic acid, benzyl and t-butyl and for cysteine, triphenylmethyl (trityl).

10 In the solid phase peptide synthesis method, the  $\alpha$ -C-terminal amino acid is attached to a suitable solid support or resin. Suitable solid supports useful for the above synthesis are those materials which are inert to the reagents and reaction conditions of the stepwise condensation-deprotection reactions, as well as being insoluble in the media used. The preferred solid support for synthesis of  $\alpha$ -C-terminal carboxy peptides is 4-hydroxymethylphenoxymethyl-copoly(styrene-1%  
15 divinylbenzene). The preferred solid support for  $\alpha$ -C-terminal amide peptides is the 4-(2',4'-dimethoxyphenyl-Fmoc-aminomethyl)phenoxyacetamidoethyl resin available from Applied Biosystems (Foster City, Calif.). The  $\alpha$ -C-terminal amino acid is coupled to the resin by means of N,N'-dicyclohexylcarbodiimide (DCC),  
20 N,N'-diisopropylcarbodiimide (DIC) or O-benzotriazol-1-yl-N,N,N',N'-tetramethyluronium-hexafluorophosphate (HBTU), with or without 4-dimethylaminopyridine (DMAP), 1-hydroxybenzotriazole (HOBt), benzotriazol-1-yloxy-tris(dimethylamino)phosphonium-hexafluorophosphate (BOP) or bis(2-oxo-3-oxazolidinyl)phosphine chloride (BOPCl), mediated coupling for from  
25 about 1 to about 24 hours at a temperature of between 10° and 50°C in a solvent such as dichloromethane or DMF.

When the solid support is 4-(2',4'-dimethoxyphenyl-Fmoc-aminomethyl)phenoxy-acetamidoethyl resin, the Fmoc group is cleaved with a secondary amine, preferably piperidine, prior to coupling with the  $\alpha$ -C-terminal  
30 amino acid as described above. The preferred method for coupling to the

deprotected 4-(2',4'-dimethoxyphenyl-Fmoc-aminomethyl)phenoxy-  
acetamidoethyl resin is O-benzotriazol-1-yl-N,N,N',N'-  
tetramethyluroniumhexafluoro-phosphate (HBTU, 1 equiv.) and 1-  
hydroxybenzotriazole (HOBT, 1 equiv.) in DMF. The coupling of successive  
5 protected amino acids can be carried out in an automatic polypeptide synthesizer  
as is well known in the art. In a preferred embodiment, the  $\alpha$ -N-terminal amino  
acids of the growing peptide chain are protected with Fmoc. The removal of the  
Fmoc protecting group from the  $\alpha$ -N-terminal side of the growing peptide is  
accomplished by treatment with a secondary amine, preferably piperidine. Each  
10 protected amino acid is then introduced in about 3-fold molar excess, and the  
coupling is preferably carried out in DMF. The coupling agent is normally O-  
benzotriazol-1-yl-N,N,N',N'-tetramethyluroniumhexafluorophosphate (HBTU, 1  
equiv.) and 1-hydroxybenzotriazole (HOBT, 1 equiv.).

At the end of the solid phase synthesis, the polypeptide is removed from  
15 the resin and deprotected, either in successively or in a single operation. Removal  
of the polypeptide and deprotection can be accomplished in a single operation by  
treating the resin-bound polypeptide with a cleavage reagent comprising  
thioanisole, water, ethanedithiol and trifluoroacetic acid. In cases wherein the  $\alpha$ -  
C-terminal of the polypeptide is an alkylamide, the resin is cleaved by aminolysis  
20 with an alkylamine. Alternatively, the peptide may be removed by  
transesterification, e.g. with methanol, followed by aminolysis or by direct  
transamidation. The protected peptide may be purified at this point or taken to the  
next step directly. The removal of the side chain protecting groups is accomplished  
using the cleavage cocktail described above. The fully deprotected peptide is  
25 purified by a sequence of chromatographic steps employing any or all of the  
following types: ion exchange on a weakly basic resin (acetate form); hydrophobic  
adsorption chromatography on underivitized polystyrene-divinylbenzene (for  
example, Amberlite XAD); silica gel adsorption chromatography; ion exchange  
chromatography on carboxymethylcellulose; partition chromatography, e.g. on  
30 Sephadex G-25, LH-20 or countercurrent distribution; high performance liquid

chromatography (HPLC), especially reverse-phase HPLC on octyl- or octadecylsilyl-silica bonded phase column packing.

Molecular weights of these ITPs are determined using Fast Atom Bombardment (FAB) Mass Spectroscopy.

5

**(1) N-Terminal Protective Groups**

As discussed above, the term "N-protecting group" refers to those groups intended to protect the  $\alpha$ -N-terminal of an amino acid or peptide or to otherwise protect the amino group of an amino acid or peptide against undesirable reactions during synthetic procedures. Commonly used N-protecting groups are disclosed in  
10 Greene, "Protective Groups In Organic Synthesis," (John Wiley & Sons, New York (1981)), which is hereby incorporated by reference. Additionally, protecting groups can be used as pro-drugs which are readily cleaved *in vivo*, for example, by enzymatic hydrolysis, to release the biologically active parent.  $\alpha$ -N-protecting  
15 groups comprise loweralkanoyl groups such as formyl, acetyl ("Ac"), propionyl, pivaloyl, t-butylacetyl and the like; other acyl groups include 2-chloroacetyl, 2-bromoacetyl, trifluoroacetyl, trichloroacetyl, phthalyl, o-nitrophenoxycarbonyl, -chlorobutyryl, benzoyl, 4-chlorobenzoyl, 4-bromobenzoyl, 4-nitrobenzoyl and the like; sulfonyl groups such as benzenesulfonyl, p-toluenesulfonyl and the like;  
20 carbamate forming groups such as benzyloxycarbonyl, p-chlorobenzyloxycarbonyl, p-methoxybenzyloxycarbonyl, p-nitrobenzyloxycarbonyl, 2-nitrobenzyloxycarbonyl, p-bromobenzyloxycarbonyl, 3,4-dimethoxybenzyloxycarbonyl, 3,5-dimethoxybenzyloxycarbonyl, 2,4-dimethoxybenzyloxycarbonyl, 4-ethoxybenzyloxycarbonyl, 2-nitro-4,5-  
25 dimethoxybenzyloxycarbonyl, 3,4,5-trimethoxybenzyloxycarbonyl, 1-(p-biphenyl)-1-methylethoxycarbonyl,  $\alpha,\alpha$ -dimethyl-3,5-dimethoxybenzyloxycarbonyl, benzhydryloxycarbonyl, t-butyloxycarbonyl (Boc), diisopropylmethoxycarbonyl, isopropylloxycarbonyl, ethoxycarbonyl, methoxycarbonyl, allyloxycarbonyl, 2,2,2-trichloroethoxycarbonyl,  
30 phenoxycarbonyl, 4-nitrophenoxycarbonyl, fluorenyl-9-methoxycarbonyl,

cyclopentyloxycarbonyl, adamantyloxycarbonyl, cyclohexyloxycarbonyl, phenylthiocarbonyl and the like; arylalkyl groups such as benzyl, triphenylmethyl, benzyloxymethyl, 9-fluorenylmethyloxycarbonyl (Fmoc) and the like and silyl groups such as trimethylsilyl and the like.

5

(2) Carboxy Protective Groups

As discussed above, the term "carboxy protecting group" refers to a carboxylic acid protecting ester or amide group employed to block or protect the carboxylic acid functionality while the reactions involving other functional sites of the compound are performed. Carboxy protecting groups are disclosed in Greene, "Protective Groups in Organic Synthesis" pp. 152-186 (1981), which is hereby incorporated by reference. Additionally, a carboxy protecting group can be used as a pro-drug whereby the carboxy protecting group can be readily cleaved *in vivo*, for example by enzymatic hydrolysis, to release the biologically active parent.

Such carboxy protecting groups are well known to those skilled in the art, having been extensively used in the protection of carboxyl groups in the penicillin and cephalosporin fields as described in U.S. Pat. Nos. 3,840,556 and 3,719,667, the disclosures of which are hereby incorporated herein by reference. Representative carboxy protecting groups are C<sub>1</sub> -C<sub>8</sub> loweralkyl (e.g., methyl, ethyl or t-butyl and the like); arylalkyl such as phenethyl or benzyl and substituted derivatives thereof such as alkoxybenzyl or nitrobenzyl groups and the like; arylalkenyl such as phenylethenyl and the like; aryl and substituted derivatives thereof such as 5-indanyl and the like; dialkylaminoalkyl such as dimethylaminoethyl and the like); alkanoyloxyalkyl groups such as acetoxymethyl, butyryloxymethyl, valeryloxymethyl, isobutyryloxymethyl, isovaleryloxymethyl, 1-(propionyloxy)-1-ethyl, 1-(pivaloyloxy)-1-ethyl, 1-methyl-1-(propionyloxy)-1-ethyl, pivaloyloxymethyl, propionyloxymethyl and the like; cycloalkanoyloxyalkyl groups such as cyclopropylcarbonyloxymethyl, cyclobutylcarbonyloxymethyl, cyclopentylcarbonyloxymethyl, cyclohexylcarbonyloxymethyl and the like; aroyloxyalkyl such as benzoyloxymethyl, benzoyloxyethyl and the like;

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arylalkylcarbonyloxyalkyl such as benzylcarbonyloxymethyl, 2-benzylcarbonyloxyethyl and the like; alkoxycarbonylalkyl or cycloalkyloxycarbonylalkyl such as methoxycarbonylmethyl, cyclohexyloxycarbonylmethyl, 1-methoxycarbonyl-1-ethyl and the like;

5 alkoxycarbonyloxyalkyl or cycloalkyloxycarbonyloxyalkyl such as methoxycarbonyloxymethyl, t-butyloxycarbonyloxymethyl, 1-ethoxycarbonyloxy-1-ethyl, 1-cyclohexyloxycarbonyloxy-1-ethyl and the like;

aryloxycarbonyloxyalkyl such as 2-(phenoxycarbonyloxy)ethyl, 2-(5-indanyloxycarbonyloxy)ethyl and the like; alkoxylalkylcarbonyloxyalkyl such as 2-

10 (1-methoxy-2-methylpropan-2-oyloxy)ethyl and like;

arylalkyloxycarbonyloxyalkyl such as 2-(benzyloxycarbonyloxy)ethyl and the like;

arylalkenyloxycarbonyloxyalkyl such as 2-(3-phenylpropen-2-yloxycarbonyloxy)ethyl and the like; alkoxycarbonylaminoalkyl such as t-

butyloxycarbonylaminomethyl and the like; alkylaminocarbonylaminoalkyl such

15 as methylaminocarbonylaminomethyl and the like; alkanoylaminoalkyl such as acetylaminomethyl and the like; heterocycliccarbonyloxyalkyl such as 4-methylpiperazinylcarbonyloxymethyl and the like; dialkylaminocarbonylalkyl

such as dimethylaminocarbonylmethyl, diethylaminocarbonylmethyl and the like;

(5-(loweralkyl)-2-oxo-1,3-dioxolen-4-yl)alkyl such as (5-t-butyl-2-oxo-1,3-

20 dioxolen-4-yl)methyl and the like; and (5-phenyl-2-oxo-1,3-dioxolen-4-yl)alkyl

such as (5-phenyl-2-oxo-1,3-dioxolen-4-yl)methyl and the like.

Representative amide carboxy protecting groups are aminocarbonyl and loweralkylaminocarbonyl groups.

25 Preferred carboxy-protected compounds of the invention are compounds wherein the protected carboxy group is a loweralkyl, cycloalkyl or arylalkyl ester, for example, methyl ester, ethyl ester, propyl ester, isopropyl ester, butyl ester, sec-butyl ester, isobutyl ester, amyl ester, isoamyl ester, octyl ester, cyclohexyl ester, phenylethyl ester and the like or an alkanoyloxyalkyl, cycloalkanoyloxyalkyl,

30 aroyloxyalkyl or an arylalkylcarbonyloxyalkyl ester. Preferred amide carboxy

protecting groups are loweralkylaminocarbonyl groups. For example, aspartic acid may be protected at the  $\alpha$ -C-terminal by an acid labile group (e.g. t-butyl) and protected at the  $\beta$ -C-terminal by a hydrogenation labile group (e.g. benzyl) then deprotected selectively during synthesis.

5

#### **B. Peptide Modification**

The manner of producing the modified peptides of the present invention will vary widely, depending upon the nature of the various elements comprising the peptide. The synthetic procedures will be selected so as to be simple, provide  
10 for high yields, and allow for a highly purified stable product. Normally, the chemically reactive group will be created at the last stage of the synthesis, for example, with a carboxyl group, esterification to form an active ester. Specific methods for the production of modified peptides of the present invention are described below.

15 Specifically, the selected peptide is first assayed for anti-viral activity, and then is modified with the linking group only at either the N-terminus, C-terminus or interior of the peptide. The anti-viral activity of this modified peptide-linking group is then assayed. If the anti-viral activity is not reduced dramatically (i.e., reduced less than 10-fold), then the stability of the modified peptide-linking group  
20 is measured by its *in vivo* lifetime. If the stability is not improved to a desired level, then the peptide is modified at an alternative site, and the procedure is repeated until a desired level of anti-viral and stability is achieved.

More specifically, each peptide selected to undergo modification with a linker and a reactive entity group will be modified according to the following  
25 criteria: if a terminal carboxylic group is available on the peptide and is not critical for the retention of anti-viral activity, and no other sensitive functional group is present on the peptide, then the carboxylic acid will be chosen as attachment point for the linker-reactive group modification. If the terminal carboxylic group is involved in anti-viral activity, or if no carboxylic acids are available, then any  
30 other sensitive functional group not critical for the retention of anti-viral activity

will be selected as the attachment point for the linker-reactive entity modification. If several sensitive functional groups are available on a peptide, a combination of protecting groups will be used in such a way that after addition of the linker/reactive entity and deprotection of all the protected sensitive functional groups, retention of anti-viral activity is still obtained. If no sensitive functional groups are available on the peptide, or if a simpler modification route is desired, synthetic efforts will allow for a modification of the original peptide in such a way that retention of anti-viral is maintained. In this case the modification will occur at the opposite end of the peptide

An NHS derivative may be synthesized from a carboxylic acid in absence of other sensitive functional groups in the peptide. Specifically, such a peptide is reacted with N-hydroxysuccinimide in anhydrous  $\text{CH}_2\text{Cl}_2$  and EDC, and the product is purified by chromatography or recrystallized from the appropriate solvent system to give the NHS derivative.

Alternatively, an NHS derivative may be synthesized from a peptide that contains an amino and/or thiol group and a carboxylic acid. When a free amino or thiol group is present in the molecule, it is preferable to protect these sensitive functional groups prior to perform the addition of the NHS derivative. For instance, if the molecule contains a free amino group, a transformation of the amine into a Fmoc or preferably into a tBoc protected amine is necessary prior to perform the chemistry described above. The amine functionality will not be deprotected after preparation of the NHS derivative. Therefore this method applies only to a compound whose amine group is not required to be freed to induce the desired anti-viral effect. If the amino group needs to be freed to retain the original properties of the molecule, then another type of chemistry described below has to be performed.

In addition, an NHS derivative may be synthesized from a peptide containing an amino or a thiol group and no carboxylic acid. When the selected molecule contains no carboxylic acid, an array of bifunctional linkers can be used to convert the molecule into a reactive NHS derivative. For instance, ethylene



glycol-bis(succinimidydsuccinate) (EGS) and triethylamine dissolved in DMF and added to the free amino containing molecule (with a ratio of 10:1 in favor of EGS) will produce the mono NHS derivative. To produce an NHS derivative from a thiol derivatized molecule, one can use N-[ $\gamma$ -maleimidobutyryloxy]succinimide ester (GMBS) and triethylamine in DMF. The maleimido group will react with the free thiol and the NHS derivative will be purified from the reaction mixture by chromatography on silica or by HPLC.

An NHS derivative may also be synthesized from a peptide containing multiple sensitive functional groups. Each case will have to be analyzed and solved in a different manner. However, thanks to the large array of protecting groups and bifunctional linkers that are commercially available, this invention is applicable to any peptide with preferably one chemical step only to modify the peptide (as described above) or two steps (as described above involving prior protection of a sensitive group) or three steps (protection, activation and deprotection). Under exceptional circumstances only, would multiple steps (beyond three steps) synthesis be required to transform a peptide into an active NHS or maleimide derivative.

A maleimide derivative may also be synthesized from a peptide containing a free amino group and a free carboxylic acid. To produce a maleimide derivative from a amino derivatized molecule, one can use N-[ $\gamma$ -maleimidobutyryloxy]succinimide ester (GMBS) and triethylamine in DMF. The succinimide ester group will react with the free amino and the maleimide derivative will be purified from the reaction mixture by crystallization or by chromatography on silica or by HPLC.

Finally, a maleimide derivative may be synthesized from a peptide containing multiple other sensitive functional groups and no free carboxylic acids.

When the selected molecule contains no carboxylic acid, an array of bifunctional crosslinking reagents can be used to convert the molecule into a reactive NHS derivative. For instance maleimidopropionic acid (MPA) can be coupled to the free amine to produce a maleimide derivative through reaction of the free amine

with the carboxylic group of MPA using HBTU/HOBt/DIEA activation in DMF.

Many other commercially available heterobifunctional crosslinking reagents can alternatively be used when needed. A large number of bifunctional compounds are available for linking to entities. Illustrative reagents include:

5 azidobenzoyl hydrazide, N-[4-(p-azidosalicylamino)butyl]-3'-[2'-pyridyldithio)propionamide), bis-sulfosuccinimidyl suberate, dimethyl adipimide, disuccinimidyl tartrate, N-y-maleimidobutyryloxysuccinimide ester, N-hydroxy sulfosuccinimidyl-4-azidobenzoate, N-succinimidyl [4-azidophenyl]-1,3'-dithiopropionate, N-succinimidyl [4-iodoacetyl]aminobenzoate,

10 glutaraldehyde, and succinimidyl 4-[N-maleimidomethyl]cyclohexane-1-carboxylate.

#### 6. Uses of Modified Anti-Viral Peptides

Modified anti-viral peptides of the invention may be used as a therapeutic agent in the treatment of patients who are suffering from viral infection, and can be administered to patients according to the methods described below and other methods known in the art. Effective therapeutic dosages of the modified peptides may be determined through procedures well known by those in the art and will take into consideration any concerns over potential toxicity of the peptide.

15

The modified peptides can also be administered prophylactically to previously uninfected individuals. This can be advantageous in cases where an individual has been subjected to a high risk of exposure to a virus, as can occur when individual has been in contact with an infected individual where there is a high risk of viral transmission. This can be especially advantageous where there is known cure for the virus, such as the HIV virus. As a example, prophylactic administration of a modified anti-HIV peptide would be advantageous in a situation where a health care worker has been exposed to blood from an HIV-infected individual, or in other situations where an individual engaged in high-risk activities that potentially expose that individual to the HIV virus.

20

25

30

7. Administration of Modified Anti-Viral and Anti-Fusogenic Peptides

Generally, the modified peptides will be administered in a physiologically acceptable medium, e.g. deionized water, phosphate buffered saline (PBS), saline, aqueous ethanol or other alcohol, plasma, proteinaceous solutions, mannitol, aqueous glucose, alcohol, vegetable oil, or the like. Other additives which may be included include buffers, where the media are generally buffered at a pH in the range of about 5 to 10, where the buffer will generally range in concentration from about 50 to 250 mM, salt, where the concentration of salt will generally range from about 5 to 500 mM, physiologically acceptable stabilizers, and the like. The compositions may be lyophilized for convenient storage and transport.

The subject modified peptides will for the most part be administered parenterally, such as intravenously (IV), intraarterially (IA), intramuscularly (IM), subcutaneously (SC), or the like. Administration may in appropriate situations be by transfusion. In some instances, where reaction of the functional group is relatively slow, administration may be oral, nasal, rectal, transdermal or aerosol, where the nature of the conjugate allows for transfer to the vascular system. Usually a single injection will be employed although more than one injection may be used, if desired. The modified peptides may be administered by any convenient means, including syringe, trocar, catheter, or the like.

The particular manner of administration will vary depending upon the amount to be administered, whether a single bolus or continuous administration, or the like. Preferably, the administration will be intravascularly, where the site of introduction is not critical to this invention, preferably at a site where there is rapid blood flow, e.g., intravenously, peripheral or central vein. Other routes may find use where the administration is coupled with slow release techniques or a protective matrix. The intent is that the modified peptide be effectively distributed in the blood, so as to be able to react with the blood components. The concentration of the conjugate will vary widely, generally ranging from about 1 pg/ml to 50 mg/ml. The total administered intravascularly will generally be in the range of about 0.1 mg/ml to about 10 mg/ml, more usually about 1 mg/ml to about

5 mg/ml.

By bonding to long-lived components of the blood, such as immunoglobulin, serum albumin, red blood cells and platelets, a number of advantages ensue. The activity of the peptide is extended for days to weeks. Only one administration need be given during this period of time. Greater specificity can be achieved, since the active compound will be primarily bound to large molecules, where it is less likely to be taken up intracellularly to interfere with other physiological processes.

## 8. Monitoring the Presence of Modified Peptides

The blood of the mammalian host may be monitored for the presence of the modified peptide compound one or more times. By taking a portion or sample of the blood of the host, one may determine whether the peptide has become bound to the long-lived blood components in sufficient amount to be therapeutically active and, thereafter, the level of the peptide compound in the blood. If desired, one may also determine to which of the blood components the peptide is bound. This is particularly important when using non-specific modified peptides. For specific maleimide-modified peptides, it is much simpler to calculate the half life of serum albumin and IgG.

### A. Immuno Assays

Another aspect of this invention relates to methods for determining the concentration of the anti-viral peptides and/or analogs, or their derivatives and conjugates in biological samples (such as blood) using antibodies specific for the peptides, peptide analogs or their derivatives and conjugates, and to the use of such antibodies as a treatment for toxicity potentially associated with such peptides, analogs, and/or their derivatives or conjugates. This is advantageous because the increased stability and life of the peptides in vivo in the patient might lead to novel problems during treatment, including increased possibility for toxicity.

The use of anti-therapeutic agent antibodies, either monoclonal or

polyclonal, having specificity for a particular peptide, peptide analog or derivative thereof, can assist in mediating any such problem. The antibody may be generated or derived from a host immunized with the particular peptide, analog or derivative thereof, or with an immunogenic fragment of the agent, or a synthesized immunogen corresponding to an antigenic determinant of the agent. Preferred antibodies will have high specificity and affinity for native, modified and conjugated forms of the peptide, peptide analog or derivative. Such antibodies can also be labeled with enzymes, fluorochromes, or radiolables.

Antibodies specific for modified peptides may be produced by using purified peptides for the induction of peptide-specific antibodies. By induction of antibodies, it is intended not only the stimulation of an immune response by injection into animals, but analogous steps in the production of synthetic antibodies or other specific binding molecules such as screening of recombinant immunoglobulin libraries. Both monoclonal and polyclonal antibodies can be produced by procedures well known in the art.

The anti-peptide antibodies may be used to treat toxicity induced by administration of the modified peptide, analog or derivative thereof, and may be used ex vivo or in vivo. Ex vivo methods would include immuno-dialysis treatment for toxicity employing anti-therapeutic agent antibodies fixed to solid supports. In vivo methods include administration of anti-therapeutic agent antibodies in amounts effective to induce clearance of antibody-agent complexes.

The antibodies may be used to remove the modified peptides, analogs or derivatives thereof, and conjugates thereof, from a patient's blood ex vivo by contacting the blood with the antibodies under sterile conditions. For example, the antibodies can be fixed or otherwise immobilized on a column matrix and the patient's blood can be removed from the patient and passed over the matrix. The modified peptide, peptide analogs, derivatives or conjugates will bind to the antibodies and the blood containing a low concentration of peptide, analog, derivative or conjugate, then may be returned to the patient's circulatory system. The amount of peptide compound removed can be controlled by adjusting the

pressure and flow rate.

Preferential removal of the peptides, analogs, derivatives and conjugates from the plasma component of a patient's blood can be effected, for example, by the use of a semipermeable membrane, or by otherwise first separating the plasma component from the cellular component by ways known in the art prior to passing the plasma component over a matrix containing the anti-therapeutic antibodies. Alternatively the preferential removal of peptide-conjugated blood cells, including red blood cells, can be effected by collecting and concentrating the blood cells in the patient's blood and contacting those cells with fixed anti-therapeutic antibodies to the exclusion of the serum component of the patient's blood.

The anti-therapeutic antibodies can be administered in vivo, parenterally, to a patient that has received the peptide, analogs, derivatives or conjugates for treatment. The antibodies will bind peptide compounds and conjugates. Once bound the peptide activity will be hindered if not completely blocked thereby reducing the biologically effective concentration of peptide compound in the patient's bloodstream and minimizing harmful side effects. In addition, the bound antibody-peptide complex will facilitate clearance of the peptide compounds and conjugates from the patient's blood stream.

The invention having been fully described can be further appreciated and understood with reference to the following non-limiting examples.

### Example 1

#### Preparation of a Modified DP 178 --Synthesis of

**YTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWFK(MPA)-NH<sub>2</sub>**

5           In this example, DP178 (SEQ ID NO:1) is synthesized and modified to include a linker and maleimide group according to the following synthesis scheme. As reported in U.S. Pat. Nos. 6,013,236 and 6,020,459, DP178 is a potent inhibitor of HIV-1, and inhibits both cell-induced syncytia formation between HIV-1 infected and uninfected cells and infection of uninfected cells by cell-free HIV-1 virus.

10           Solid phase peptide synthesis of the modified peptide on a 100 μmole scale is performed using manual solid-phase synthesis, a Symphony Peptide Synthesizer and Fmoc protected Rink Amide MBHA. The following protected amino acids are sequentially added to resin: Fmoc-Lys(Aloc)-OH, Fmoc-Phe-OH, Fmoc-Trp(Boc)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Leu-OH, Fmoc-Ser(tBu)-OH, Fmoc-Ala-OH, Fmoc-Trp(Boc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Asp(tBu)-OH, Fmoc-Leu-OH, Fmoc-Glu(tBu)-OH, Fmoc-Leu-OH, Fmoc-Leu-OH, Fmoc-Glu(tBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Met-OH, Fmoc-Lys(Boc)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Glu(tBu)-OH; Fmoc-Gln(Trt)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Ile-OH, Fmoc-Leu-OH, Fmoc-Ser(tBu)-OH, Fmoc-His(Boc)-OH, Fmoc-Ile-OH, Fmoc-Leu-OH, Fmoc-Ser(tBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Tyr(tBu)-OH. They are dissolved in *N,N*-dimethylformamide (DMF) and, according to the sequence, activated using *O*-benzotriazol-1-yl-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU) and Diisopropylethylamine (DIEA). Removal of the Fmoc protecting group is achieved using a solution of 20% (V/V) piperidine in *N,N*-dimethylformamide (DMF) for 20 minutes (step 1). At the end of the

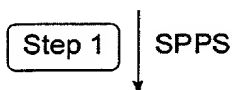
synthesis. The selective deprotection of the Lys (Aloc) group is performed manually and accomplished by treating the resin with a solution of 3 eq of  $\text{Pd(PPh}_3)_4$  dissolved in 5 mL of  $\text{CHCl}_3$ :NMM:HOAc (18:1:0.5) for 2 h (Step 2). The resin is then washed with  $\text{CHCl}_3$  (6 x 5 mL), 20% HOAc in DCM (6 x 5 mL),  
5 DCM (6 x 5 mL), and DMF (6 x 5 mL). The synthesis is then re-automated for the addition of the 3-maleimidopropionic acid (Step 3). Between every coupling, the resin is washed 3 times with *N,N*-dimethylformamide (DMF) and 3 times with isopropanol. The peptide is cleaved from the resin using 85% TFA/5% TIS/5% thioanisole and 5% phenol, followed by precipitation by dry-ice cold  $\text{Et}_2\text{O}$  (Step  
10 4). The product is purified by preparative reversed phased HPLC using a Varian (Rainin) preparative binary HPLC system: gradient elution of 30-55% B (0.045% TFA in  $\text{H}_2\text{O}$  (A) and 0.045% TFA in  $\text{CH}_3\text{CN}$  (B)) over 180 min at 9.5 mL/min using a Phenomenex Luna 10  $\mu$  phenyl-hexyl, 21 mm x 25 cm column and UV detector (Varian Dynamax UVD II) at  $\lambda$ 214 and 254 nm to afford the desired  
15 modified peptide (i.e., DAC) in >95% purity, as determined by RP-HPLC.

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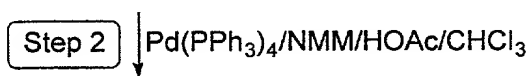


# DP-178 C

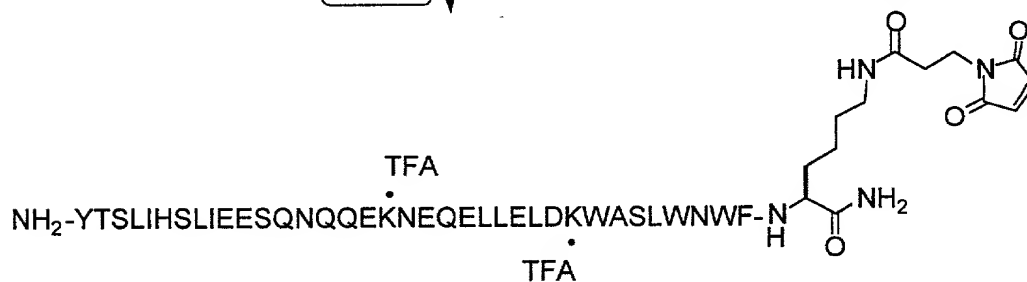
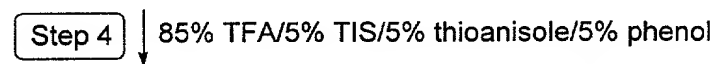
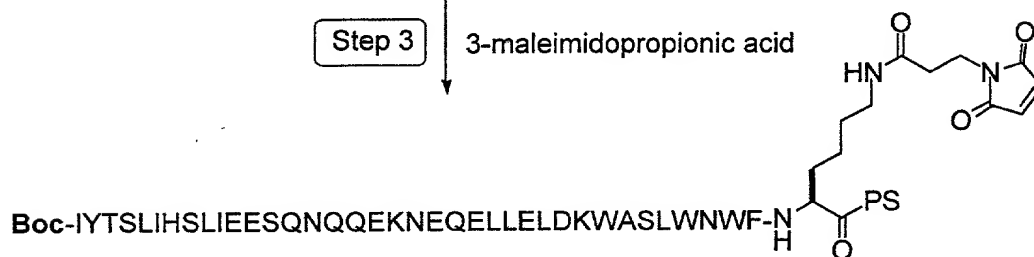
Fmoc-Rink Amide MBHA Resin



Boc-YTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWF-Lys(Aloc)-PS



Boc-YTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWF-Lys-PS



## Example 2

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Preparation of a Modified DP107--Synthesis of  
NNLLRAIEAQQHLLQLTVWQIKQLQARILAVEYLKDQK(MPA)NH<sub>2</sub>

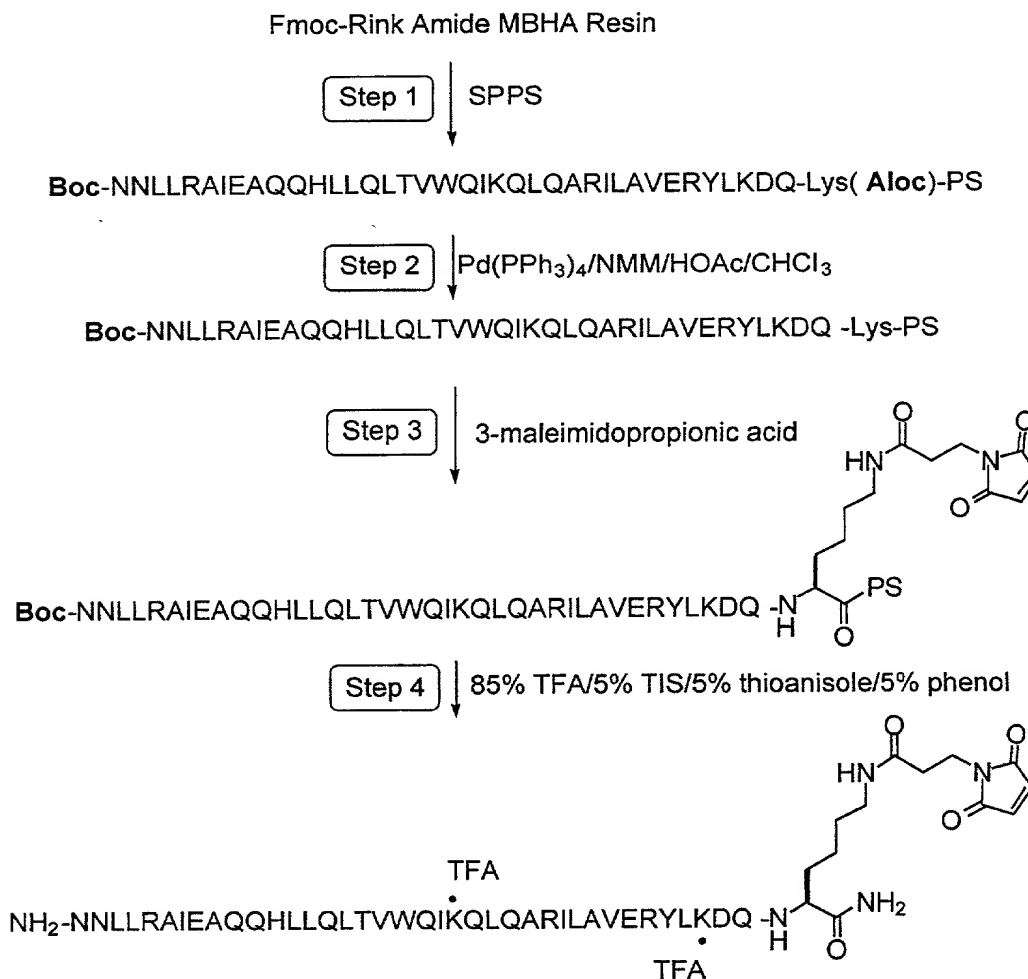
In this example, DP107 (SEQ ID NO:2) is synthesized and modified to

include a linker and maleimide group according to the following synthesis scheme. As reported in U.S. Pat. Nos. 6,013,236 and 6,020,459, DP107 exhibits potent antiviral activity against HIV.

5           Solid phase peptide synthesis of the modified peptide on a 100  $\mu$ mole scale is performed using manual solid-phase synthesis, a Symphony Peptide Synthesizer and Fmoc protected Rink Amide MBHA. The following protected amino acids are sequentially added to resin: Fmoc-Lys(Aloc)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Asp(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Leu-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Val-OH, Fmoc-Ala-OH, Fmoc-Leu-OH, Fmoc-Ile-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Ala-OH, Fmoc-Gln(Trt)-OH, Fmoc-Leu-OH, Fmoc-Gln(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ile-OH, Fmoc-Gln(Trt)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Val-OH, Fmoc-Thr(tBu)-OH, Fmoc-Leu-OH, Fmoc-Gln(Trt)-OH, Fmoc-Leu-OH, Fmoc-Leu-OH, Fmoc-His(Boc)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Ala-OH, Fmoc-Glu(tBu)-OH, Fmoc-Ile-OH, Fmoc-Ala-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Leu-OH, Fmoc-Leu-OH, Fmoc-Asn(Trt)-OH, Fmoc-Asn(Trt)-OH, They are dissolved in *N,N*-dimethylformamide (DMF) and, according to the sequence, activated using *O*-benzotriazol-1-yl-*N,N,N',N'*-tetramethyl-uronium hexafluorophosphate (HBTU) and Diisopropylethylamine (DIEA). Removal of the Fmoc protecting group is achieved using a solution of 20% (V/V) piperidine in *N,N*-dimethylformamide (DMF) for 20 minutes (step 1). At the end of the synthesis. The selective deprotection of the Lys (Aloc) group is performed manually and accomplished by treating the resin with a solution of 3 eq of Pd(PPh<sub>3</sub>)<sub>4</sub> dissolved in 5 mL of CHCl<sub>3</sub>:NMM:HOAc (18:1:0.5) for 2 h (Step 2). The resin is then washed with CHCl<sub>3</sub> (6 x 5 mL), 20% HOAc in DCM (6 x 5 mL), DCM (6 x 5 mL), and DMF (6 x 5 mL). The synthesis is then re-automated for the addition of the 3-maleimidopropionic acid (Step 3). Between every coupling, the resin is washed 3 times with *N,N*-dimethylformamide (DMF) and 3 times with isopropanol. The peptide is cleaved from the resin using 85% TFA/5% TIS/5% thioanisole and 5% phenol, followed by precipitation by

5

**DP-107 C**



### Example 3

#### Preparation of a Modified anti-RSV peptide (C terminal)

In this example, the peptide

5 VITIELSNIKENKCNGAKVKLIKQELDKYKNAV (SEQ ID NO:16) is modified to include a linker and maleimide group according to the synthesis scheme set forth below. As reported in U.S. Pat. Nos. 6,013,236 and 6,020,459, the native sequence (SEQ ID NO. ) inhibits viral infection of respiratory syncytial virus (RSV), including inhibiting fusion and syncytia formation between RSV-infected  
10 and uninfected Hep-2 cells.

Solid phase peptide synthesis of the modified peptide on a 100  $\mu$ mole scale is performed using manual solid-phase synthesis, a Symphony Peptide Synthesizer and Fmoc protected Rink Amide MBHA. The following protected amino acids are  
15 sequentially added to resin: Fmoc-Lys(Aloc)-OH, Fmoc-Val-OH, Fmoc-Ala-OH, Fmoc-Asn(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Asp(tBu)-OH, Fmoc-Leu-OH, Fmoc-Glu(tBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ile-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Val-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ala-OH, Fmoc-Gly-OH, Fmoc-  
20 Asn(Trt)-OH, Fmoc-Cys(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ile-OH, Fmoc-Asn(Trt)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Leu-OH, Fmoc-Glu(tBu)-OH, Fmoc-Ile-OH, Fmoc-Thr(tBu)-OH, Fmoc-Ile-OH, Fmoc-Val-OH. They are dissolved in *N,N*-dimethylformamide (DMF) and, according to the sequence, activated using *O*-benzotriazol-1-yl-*N,N,N',N'*-tetramethyl-uronium hexafluorophosphate (HBTU)  
25 and Diisopropylethylamine (DIEA). Removal of the Fmoc protecting group is achieved using a solution of 20% (V/V) piperidine in *N,N*-dimethylformamide (DMF) for 20 minutes (step 1). The selective deprotection of the Lys (Aloc) group is performed manually and accomplished by treating the resin with a solution of 3  
30 eq of Pd(PPh<sub>3</sub>)<sub>4</sub> dissolved in 5 mL of CHCl<sub>3</sub>:NMM:HOAc (18:1:0.5) for 2 h (Step

2). The resin is then washed with  $\text{CHCl}_3$  (6 x 5 mL), 20% HOAc in DCM (6 x 5 mL), DCM (6 x 5 mL), and DMF (6 x 5 mL). The synthesis is then re-automated for the addition of the 3-maleimidopropionic acid (Step 3). Between every coupling, the resin is washed 3 times with *N,N*-dimethylformamide (DMF) and 3 times with isopropanol. The peptide is cleaved from the resin using 85% TFA/5% TIS/5% thioanisole and 5% phenol, followed by precipitation by dry-ice cold  $\text{Et}_2\text{O}$  (Step 4). The product is purified by preparative reversed phased HPLC using a Varian (Rainin) preparative binary HPLC system: gradient elution of 30-55% B (0.045% TFA in  $\text{H}_2\text{O}$  (A) and 0.045% TFA in  $\text{CH}_3\text{CN}$  (B)) over 180 min at 9.5 mL/min using a Phenomenex Luna 10  $\mu$  phenyl-hexyl, 21 mm x 25 cm column and UV detector (Varian Dynamax UVD II) at  $\lambda_{214}$  and 254 nm to afford the desired modified peptide (i.e., DAC) in >95% purity, as determined by RP-HPLC.

Fmoc-Rink Amide MBHA Resin

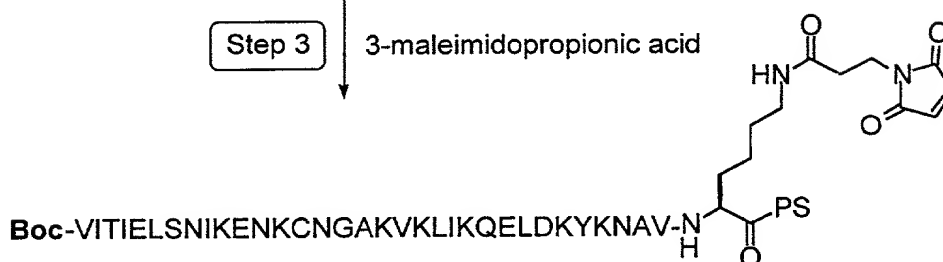
Step 1 ↓ SPPS

**Boc-VITIELSNIKENKCNGAKVKLIKQELDKYKNAV-Lys(Alloc)-PS**

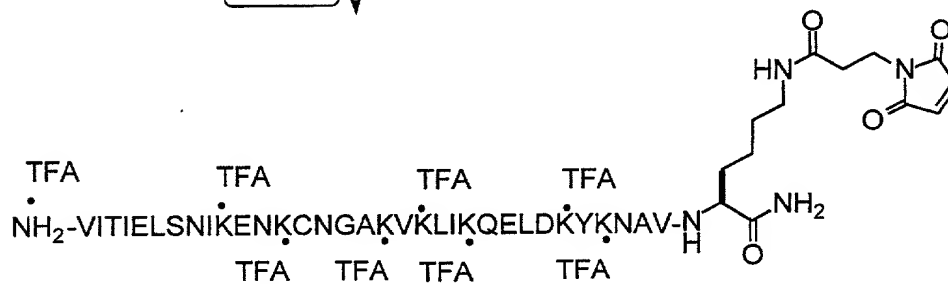
Step 2 ↓ Pd(PPh<sub>3</sub>)<sub>4</sub>/NMM/HOAc/CHCl<sub>3</sub>

**Boc-VITIELSNIKENKCNGAKVKLIKQELDKYKNAV-Lys-PS**

Step 3 ↓ 3-maleimidopropionic acid



Step 4 ↓ 85% TFA/5% TIS/5% thioanisole/5% phenol



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#### Example 4

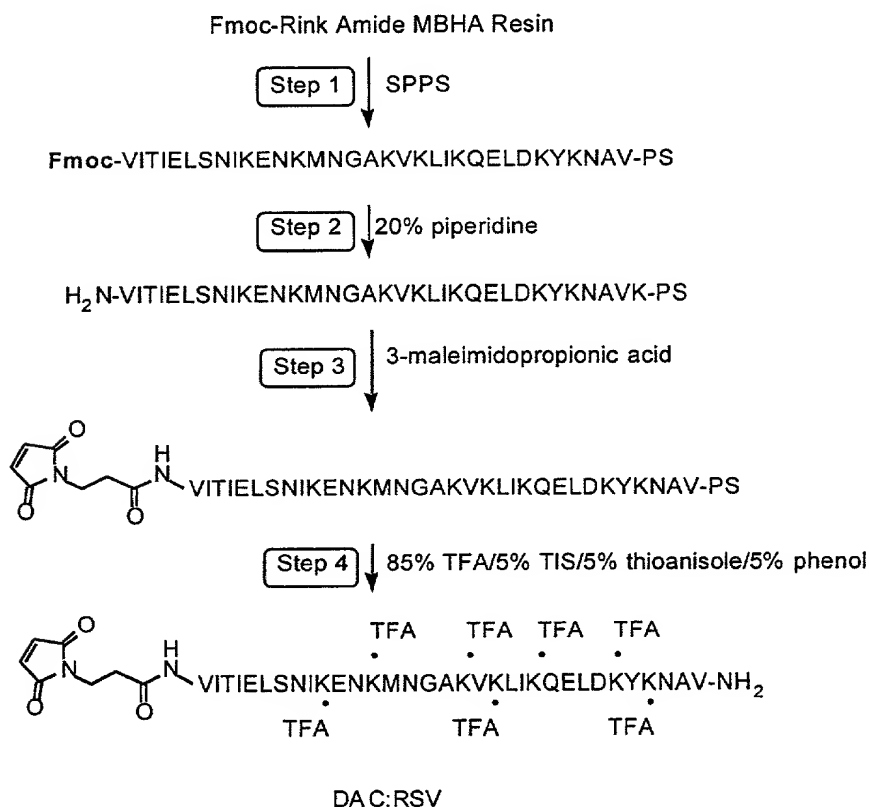
##### Preparation of a Modified anti-RSV peptide (T-N terminal)

In this example, the peptide

5 VITIELSNIKENKCNGAKVKLIKQELDKYKNAV (SEQ ID NO:17), which corresponds to the peptide of SEQ ID NO:16 but where a Cysteine (C) has been substituted for the Methionine (M), residue is synthesized and modified to include a linker and maleimide group according to the synthesis scheme set forth below. As reported in U.S. Pat. Nos. 6,013,236 and 6,020,459, the native sequence  
10 (SEQ ID NO:16) inhibits viral infection of respiratory syncytial virus (RSV), including inhibiting fusion and syncytia formation between RSV-infected and uninfected Hep-2 cells.

15 Solid phase peptide synthesis of the modified peptide on a 100  $\mu$ mole scale is performed using manual solid-phase synthesis, a Symphony Peptide Synthesizer and Fmoc protected Rink Amide MBHA. The following protected amino acids are sequentially added to resin: Fmoc-Val-OH, Fmoc-Ala-OH, Fmoc-Asn(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Asp(tBu)-  
20 OH, Fmoc-Leu-OH, Fmoc-Glu(tBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ile-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Val-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ala-OH, Fmoc-Gly-OH, Fmoc-Asn(Trt)-OH, **Fmoc-Met-OH**, Fmoc-Lys(Boc)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ile-OH, Fmoc-Asn(Trt)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Leu-  
25 OH, Fmoc-Glu(tBu)-OH, Fmoc-Ile-OH, Fmoc-Thr(tBu)-OH, Fmoc-Ile-OH, Fmoc-Val-OH. They are dissolved in *N,N*-dimethylformamide (DMF) and, according to the sequence, activated using *O*-benzotriazol-1-yl-*N,N,N,N*-tetramethyl-uronium hexafluorophosphate (HBTU) and Diisopropylethylamine (DIEA). Removal of the Fmoc protecting group is achieved using a solution of  
30 20% (V/V) piperidine in *N,N*-dimethylformamide (DMF) for 20 minutes (step 1). The synthesis is then re-automated for the addition of the 3-maleimidopropionic

acid (Step 3). Between every coupling, the resin is washed 3 times with *N,N*-dimethylformamide (DMF) and 3 times with isopropanol. The peptide is cleaved from the resin using 85% TFA/5% TIS/5% thioanisole and 5% phenol, followed by precipitation by dry-ice cold Et<sub>2</sub>O (Step 4). The product is purified by preparative reversed phased HPLC using a Varian (Rainin) preparative binary HPLC system: gradient elution of 30-55% B (0.045% TFA in H<sub>2</sub>O (A) and 0.045% TFA in CH<sub>3</sub>CN (B)) over 180 min at 9.5 mL/min using a Phenomenex Luna 10 μ phenyl-hexyl, 21 mm x 25 cm column and UV detector (Varian Dynamax UVD II) at λ<sub>214</sub> and 254 nm to afford the desired modified peptide (i.e., DAC) in >95% purity, as determined by RP-HPLC.





**Example 5**  
**Preparation of a Modified anti-RSV peptide**

In this example, the peptide SEQ ID NO:14 is synthesized and modified to include a linker and maleimide group according to the synthesis scheme set forth below. As reported in U.S. Pat. Nos. 6,013,236 and 6,020,459, SEQ ID NO:14 inhibits viral infection of respiratory syncytial virus (RSV), including inhibiting fusion and syncytia formation between RSV-infected and uninfected Hep-2 cells.

Solid phase peptide synthesis of the modified peptide on a 100  $\mu$ mole scale is performed using manual solid-phase synthesis, a Symphony Peptide Synthesizer and Fmoc protected Rink Amide MBHA. The following protected amino acids are sequentially added to resin: Fmoc-Lys(Aloc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Asp(tBu)-OH, Fmoc-Leu-OH, Fmoc-Glu(tBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ile-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Val-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ala-OH, Fmoc-Gly-OH, Fmoc-Asn(Trt)-OH, Fmoc-Cys(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ile-OH, Fmoc-Asn(Trt)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Leu-OH, Fmoc-Glu(tBu)-OH, Fmoc-Ile-OH, Fmoc-Thr(tBu)-OH, Fmoc-Ile-OH, Fmoc-Val-OH, Fmoc-Ser(tBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Tyr(tBu)-OH. They are dissolved in *N,N*-dimethylformamide (DMF) and, according to the sequence, activated using *O*-benzotriazol-1-yl-*N,N,N,N*-tetramethyl-uronium hexafluorophosphate (HBTU) and Diisopropylethylamine (DIEA). Removal of the Fmoc protecting group is achieved using a solution of 20% (V/V) piperidine in *N,N*-dimethylformamide (DMF) for 20 minutes (step 1). The selective deprotection of the Lys (Aloc) group is performed manually and accomplished by treating the resin with a solution of 3 eq of Pd(PPh<sub>3</sub>)<sub>4</sub> dissolved in 5 mL of CHCl<sub>3</sub>:NMM:HOAc (18:1:0.5) for 2 h (Step 2). The resin is then washed with CHCl<sub>3</sub> (6 x 5 mL), 20% HOAc in DCM (6 x 5 mL), DCM (6 x 5 mL), and DMF (6 x 5 mL). The synthesis is then re-automated

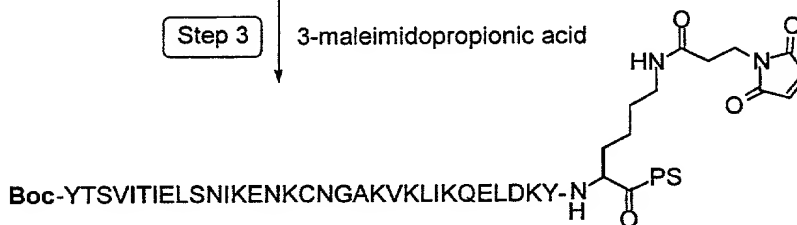
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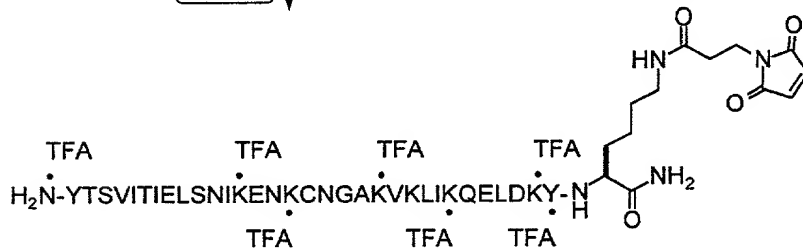
Step 1 | SPSS

**Step 2** |  $\text{Pd}(\text{PPh}_3)_4/\text{NMM}/\text{HOAc}/\text{CHCl}_3$

Step 3 3-maleimidopropionic acid



**Step 4** | 85% TFA/5% TIS/5% thioanisole/5% phenol



**Example 6 (T-143)**

**Preparation of a Modified anti-RSV peptide**

In this example, the peptide SEQ ID NO:15 is synthesized and modified to include a linker and maleimide group according to the synthesis scheme set forth below. As reported in U.S. Pat. Nos. 6,013,236 and 6,020,459, SEQ ID NO:15 inhibits viral infection of respiratory syncytial virus (RSV), including inhibiting fusion and syncytia formation between RSV-infected and uninfected Hep-2 cells.

Solid phase peptide synthesis of the modified peptide analog on a 100  $\mu$ mole scale is performed using manual solid-phase synthesis, a Symphony Peptide Synthesizer and Fmoc protected Rink Amide MBHA. The following protected amino acids are sequentially added to resin: Fmoc-Lys(Aloc)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Asp(tBu)-OH, Fmoc-Leu-OH, Fmoc-Glu(tBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ile-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Val-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ala-OH, Fmoc-Gly-OH, Fmoc-Asn(Trt)-OH, Fmoc-Cys(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ile-OH, Fmoc-Asn(Trt)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Leu-OH, Fmoc-Glu(tBu)-OH, Fmoc-Ile-OH, Fmoc-Thr(tBu)-OH, Fmoc-Ile-OH, Fmoc-Val-OH, Fmoc-Ser(tBu)-OH, Fmoc-Thr(tBu)-OH. They are dissolved in *N,N*-dimethylformamide (DMF) and, according to the sequence, activated using *O*-benzotriazol-1-yl-*N,N,N',N'*-tetramethyl-uronium hexafluorophosphate (HBTU) and Diisopropylethylamine (DIEA). Removal of the Fmoc protecting group is achieved using a solution of 20% (V/V) piperidine in *N,N*-dimethylformamide (DMF) for 20 minutes (step 1). The selective deprotection of the Lys (Aloc) group is performed manually and accomplished by treating the resin with a solution of 3 eq of Pd(PPh<sub>3</sub>)<sub>4</sub> dissolved in 5 mL of CHCl<sub>3</sub>:NMM:HOAc (18:1:0.5) for 2 h (Step 2). The resin is then washed with CHCl<sub>3</sub> (6 x 5 mL), 20% HOAc in DCM (6 x 5 mL), DCM (6 x 5 mL), and DMF (6

x 5 mL). The synthesis is then re-automated for the addition of the 3-maleimidopropionic acid (Step 3). Between every coupling, the resin is washed 3 times with *N,N*-dimethylformamide (DMF) and 3 times with isopropanol. The peptide is cleaved from the resin using 85% TFA/5% TIS/5% thioanisole and 5% phenol, followed by precipitation by dry-ice cold Et<sub>2</sub>O (Step 4). The product is purified by preparative reversed phased HPLC using a Varian (Rainin) preparative binary HPLC system: gradient elution of 30-55% B (0.045% TFA in H<sub>2</sub>O (A) and 0.045% TFA in CH<sub>3</sub>CN (B)) over 180 min at 9.5 mL/min using a Phenomenex Luna 10 μ phenyl-hexyl, 21 mm x 25 cm column and UV detector (Varian Dynamax UVD II) at λ<sub>214</sub> and 254 nm to afford the desired modified peptide (i.e., DAC) in >95% purity, as determined by RP-HPLC.

Step 1 ↓ SPPS

Step 2  $\downarrow$  Pd(PPh<sub>3</sub>)<sub>4</sub>/NMM/HOAc/CHCl<sub>3</sub>

Step 3

3-maleimidopropionic acid

Chemical structure of the conjugate: A peptide sequence (SNIKENKCNGAKVKLIKQELDKYKN) is shown, with a maleimide group attached to the C-terminus via a linker (PS).

Step 4 ↓ 85% TFA/5% TIS/5% thioanisole/5% phenol

NC(=O)CCCCNC(=O)CC1C=CC(=O)N1

Chemical structure of the peptide sequence: NC(=O)TSVITIELSNIKENKCNNGAKVVKLIKQELDKYKN-NH. The structure shows the peptide backbone with side chains represented by single dots. The N-terminus is a primary amide, and the C-terminus is a secondary amide linked to a 2-pyridone moiety.

## 5 Preparation of a Modified anti-RSV peptide (C Terminal)

In this example, the peptide SEQ ID NO:17), which corresponds to SEQ ID NO:16 with a cysteine (C) substituted for the Methionine (M), is synthesized and modified to include a linker and maleimide group according to the synthesis scheme set forth below. As reported in U.S. Pat. Nos. 6,013,236 and 6,020,459, the native sequence SEQ ID NO:16. inhibits viral infection of respiratory syncytial

virus (RSV), including inhibiting fusion and syncytia formation between RSV-infected and uninfected Hep-2 cells.

5 Solid phase peptide synthesis of the modified peptide on a 100  $\mu$ mole scale is performed using manual solid-phase synthesis, a Symphony Peptide Synthesizer and Fmoc protected Rink Amide MBHA. The following protected amino acids are sequentially added to resin: Fmoc-Lys(Aloc)-OH, Fmoc-Val-OH, Fmoc-Ala-OH, Fmoc-Asn(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Asp(tBu)-OH, Fmoc-Leu-OH, Fmoc-Glu(tBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ile-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Val-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ala-OH, Fmoc-Gly-OH, Fmoc-Asn(Trt)-OH, **Fmoc-Met-OH**, Fmoc-Lys(Boc)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ile-OH, Fmoc-Asn(Trt)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Leu-OH, Fmoc-Glu(tBu)-OH, Fmoc-Ile-OH, Fmoc-Thr(tBu)-OH, Fmoc-Ile-OH, Fmoc-Val-OH. They are dissolved in *N,N*-dimethylformamide (DMF) and, according to the sequence, activated using *O*-benzotriazol-1-yl-*N,N,N'*, *N'*-tetramethyl-uronium hexafluorophosphate (HBTU) and Diisopropylethylamine (DIEA). Removal of the Fmoc protecting group is achieved using a solution of 20% (V/V) piperidine in *N,N*-dimethylformamide (DMF) for 20 minutes (step 1). The selective deprotection of the Lys (Aloc) group is performed manually and accomplished by treating the resin with a solution of 3 eq of  $\text{Pd}(\text{PPh}_3)_4$  dissolved in 5 mL of  $\text{CHCl}_3$ :NMM:HOAc (18:1:0.5) for 2 h (Step 2). The resin is then washed with  $\text{CHCl}_3$  (6 x 5 mL), 20% HOAc in DCM (6 x 5 mL), DCM (6 x 5 mL), and DMF (6 x 5 mL). The synthesis is then re-automated for the addition of the 3-maleimidopropionic acid (Step 3). Between every coupling, the resin is washed 3 times with *N,N*-dimethylformamide (DMF) and 3 times with isopropanol. The peptide is cleaved from the resin using 85% TFA/5% TIS/5% thioanisole and 5% phenol, followed by precipitation by dry-ice cold  $\text{Et}_2\text{O}$  (Step 4). The product is purified by preparative reversed phased HPLC using a Varian (Rainin) preparative binary HPLC system: gradient elution of 30-55% B (0.045%

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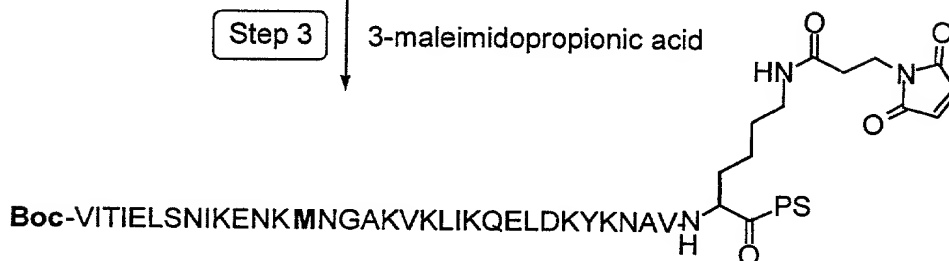
[illegible]

Step 1  $\downarrow$  SPPS

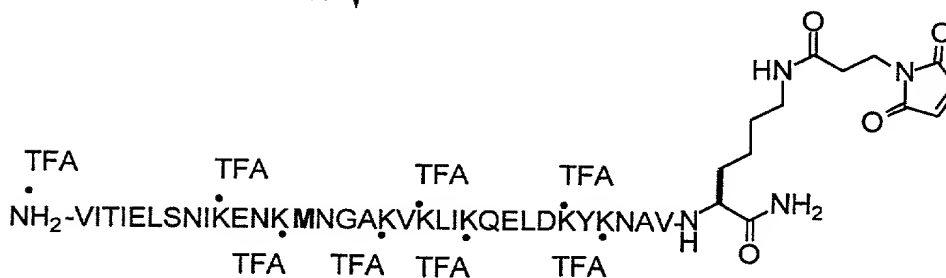
Step 2  $\downarrow$  Pd(PPh<sub>3</sub>)<sub>4</sub>/NMM/HOAc/CHCl<sub>3</sub>

Step 3

3-maleimidopropionic acid



**Step 4** ↓ 85% TFA/5% TIS/5% thioanisole/5% phenol



## 5

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Solid phase peptide synthesis of the modified peptide on a 100  $\mu$ mole scale is performed using manual solid-phase synthesis, a Symphony Peptide Synthesizer and Fmoc protected Rink Amide MBHA. The following protected amino acids are sequentially added to resin: Fmoc-Lys(Aloc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Asp(tBu)-OH, Fmoc-Ile-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Leu-OH, Fmoc-Asp(tBu)-OH, Fmoc-Leu-OH, Fmoc-Val-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Leu-OH, Fmoc-Val-OH, Fmoc-Ser(tBu)-OH, Fmoc-Val-OH, Fmoc-Gly-OH, Fmoc-Asn(Trt)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Leu-OH, Fmoc-Ser(tBu)-OH, Fmoc-Val-OH, Fmoc-Val-OH, Fmoc-Ala-OH, Fmoc-Lys(Boc)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Leu-OH, Fmoc-Leu-OH, Fmoc-Ala-OH, Fmoc-Ile-OH. They are dissolved in *N,N*-dimethylformamide (DMF) and, according to the sequence, activated using *O*-benzotriazol-1-yl-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU) and Diisopropylethylamine (DIEA). Removal of the Fmoc protecting group is achieved using a solution of 20% (V/V) piperidine in *N,N*-dimethylformamide (DMF) for 20 minutes (step 1). The selective deprotection of the Lys (Aloc) group is performed manually and accomplished by treating the resin with a solution of 3 eq of Pd(PPh<sub>3</sub>)<sub>4</sub> dissolved in 5 mL of CHCl<sub>3</sub>:NMM:HOAc (18:1:0.5) for 2 h (Step 2). The resin is then washed with CHCl<sub>3</sub> (6 x 5 mL), 20% HOAc in DCM (6 x 5 mL), DCM (6 x 5 mL), and DMF (6 x 5 mL). The synthesis is then re-automated for the addition of the 3-maleimidopropionic acid (Step 3). Between every coupling, the resin is washed 3 times with *N,N*-dimethylformamide (DMF) and 3 times with isopropanol. The peptide is cleaved from the resin using 85% TFA/5% TIS/5% thioanisole and 5% phenol, followed by precipitation by dry-ice cold Et<sub>2</sub>O (Step 4). The product is purified by preparative reversed phased HPLC using a Varian (Rainin) preparative binary HPLC system: gradient elution of 30-55% B (0.045% TFA in H<sub>2</sub>O (A) and 0.045% TFA in CH<sub>3</sub>CN (B)) over 180 min at 9.5 mL/min using a Phenomenex Luna 10  $\mu$  phenyl-hexyl, 21 mm x 25 cm column and UV detector (Varian Dynamax UVD II) at  $\lambda$  214 and 254 nm to afford the desired modified peptide

(i.e., DAC) in >95% purity, as determined by RP-HPLC.

Fmoc-Rink Amide MBHA Resin

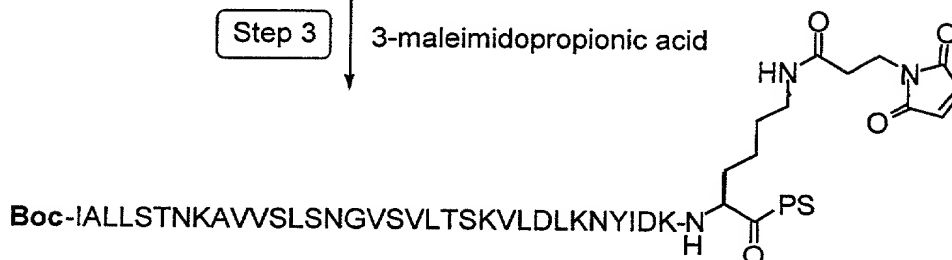
Step 1 ↓ SPPS

Boc-IALLSTNKAVVSLNSGVSVLT SKVLDLKNYIDK-Lys( Aloc)-PS

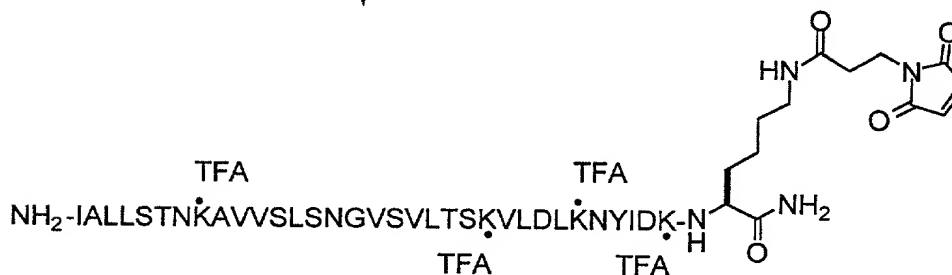
Step 2 ↓ Pd(PPh<sub>3</sub>)<sub>4</sub>/NMM/HOAc/CHCl<sub>3</sub>

Boc-IALLSTNKAVVSLNSGVSVLT SKVLDLKNYIDK-Lys -PS

Step 3 ↓ 3-maleimidopropionic acid



Step 4 ↓ 85% TFA/5% TIS/5% thioanisole/5% phenol



### Example 9 (T-173)

#### 5 Preparation of a Modified anti-HPIV peptide

In this example, the peptide SEQ ID NO:52. is synthesized and modified to include a linker and maleimide group according to the synthesis scheme set forth below. As reported in U.S. Pat. Nos. 6,013,236 and 6,020,459, SEQ ID NO:52 inhibits viral infection of human parainfluenza virus 3 (HPIV3), including inhibiting fusion and syncytia formation between HPIV3-infected Hep2 cells and

uninfected CV-1W cells.

Solid phase peptide synthesis of the modified peptide on a 100  $\mu$ mole scale is performed using manual solid-phase synthesis, a Symphony Peptide Synthesizer and Fmoc protected Rink Amide MBHA. The following protected amino acids are sequentially added to resin: Fmoc-Lys(Aloc)-OH, Fmoc-Ile-OH, Fmoc-Leu-OH, Fmoc-Asn(Trt)-OH, Fmoc-Gly-OH, Fmoc-Ile-OH, Fmoc-Ser(tBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Val-OH, Fmoc-Ser(tBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Val-OH, Fmoc-Ala-OH, Fmoc-Lys(Boc)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Asp(tBu)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Ile-OH, Fmoc-Ala-OH, Fmoc-Glu(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Ile-OH, Fmoc-Asp(tBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Ala-OH, Fmoc-Gln(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ala-OH, Fmoc-Glu(tBu)-OH, Fmoc-Val-OH. They are dissolved in *N,N*-dimethylformamide (DMF) and, according to the sequence, activated using *O*-benzotriazol-1-yl-*N,N,N',N'*-tetramethyl-uronium hexafluorophosphate (HBTU) and Diisopropylethylamine (DIEA). Removal of the Fmoc protecting group is achieved using a solution of 20% (V/V) piperidine in *N,N*-dimethylformamide (DMF) for 20 minutes (step 1). The selective deprotection of the Lys (Aloc) group is performed manually and accomplished by treating the resin with a solution of 3 eq of Pd(PPh<sub>3</sub>)<sub>4</sub> dissolved in 5 mL of CHCl<sub>3</sub>:NMM:HOAc (18:1:0.5) for 2 h (Step 2). The resin is then washed with CHCl<sub>3</sub> (6 x 5 mL), 20% HOAc in DCM (6 x 5 mL), DCM (6 x 5 mL), and DMF (6 x 5 mL). The synthesis is then re-automated for the addition of the 3-maleimidopropionic acid (Step 3). Between every coupling, the resin is washed 3 times with *N,N*-dimethylformamide (DMF) and 3 times with isopropanol. The peptide is cleaved from the resin using 85% TFA/5% TIS/5% thioanisole and 5% phenol, followed by precipitation by dry-ice cold Et<sub>2</sub>O (Step 4). The product is purified by preparative reversed phased HPLC using a Varian (Rainin) preparative binary HPLC system: gradient elution of 30-55% B (0.045% TFA in H<sub>2</sub>O (A) and 0.045% TFA in CH<sub>3</sub>CN (B)) over 180 min at 9.5 mL/min using a Phenomenex

### Fmoc-Rink Amide MBHA Resin

↓

### Step 2

$\downarrow \text{Pd(PPh}_3)_4/\text{NMM}/\text{HOAc}/\text{CHCl}_3$

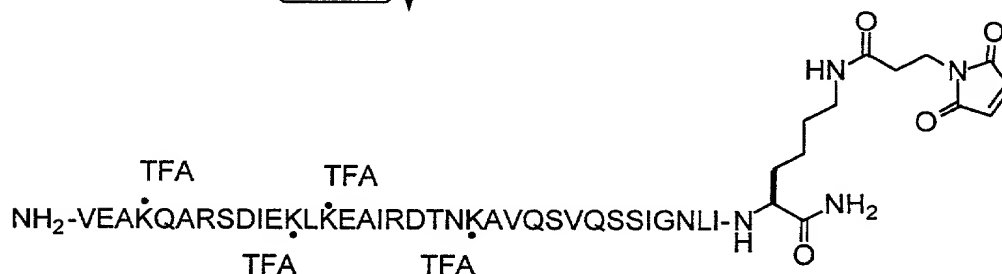
### Step 3

3-maleimidopropionic acid



### Step 4

85% TFA/5% TIS/5% thioanisole/5% phenol



### Example 10

#### Preparation of a Modified anti-HPIV peptide

In this example, the peptide SEQ ID NO:58 is synthesized and modified to include a linker and maleimide group according to the synthesis scheme set forth below. As reported in U.S. Pat. Nos. 6,013,236 and 6,020,459, SEQ ID NO:58 inhibits viral infection of human parainfluenza virus 3 (HPIV3), including inhibiting fusion and syncytia formation between HPIV3-infected Hep2 cells and uninfected CV-1W cells.

Solid phase peptide synthesis of the modified peptide on a 100  $\mu$ mole scale is performed using manual solid-phase synthesis, a Symphony Peptide Synthesizer and Fmoc protected Rink Amide MBHA. The following protected amino acids are sequentially added to resin: Fmoc-Lys(Aloc)-OH, Fmoc-Val-OH, Fmoc-Ser(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ile-OH, Fmoc-Ala-OH, Fmoc-Val-OH, Fmoc-Ile-OH, Fmoc-Leu-OH, Fmoc-Asn(Trt)-OH, Fmoc-Gly-OH, Fmoc-Ile-OH, Fmoc-Ser(tBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Val-OH, Fmoc-Ser(tBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Val-OH, Fmoc-Ala-OH, Fmoc-Lys(Boc)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Asp(tBu)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Ile-OH, Fmoc-Ala-OH, Fmoc-Glu(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Ile-OH, Fmoc-Asp(tBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Arg(Pbf)-OH. They are dissolved in *N,N*-dimethylformamide (DMF) and, according to the sequence, activated using *O*-benzotriazol-1-yl-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU) and Diisopropylethylamine (DIEA). Removal of the Fmoc protecting group is achieved using a solution of 20% (V/V) piperidine in *N,N*-dimethylformamide (DMF) for 20 minutes (step 1). The selective deprotection of the Lys (Aloc) group is performed manually and accomplished by treating the resin with a solution of 3 eq of  $\text{Pd}(\text{PPh}_3)_4$  dissolved in 5 mL of  $\text{CHCl}_3$ :NMM:HOAc (18:1:0.5) for 2 h (Step 2). The resin is then washed with

CHCl<sub>3</sub> (6 x 5 mL), 20% HOAc in DCM (6 x 5 mL), DCM (6 x 5 mL), and DMF (6 x 5 mL). The synthesis is then re-automated for the addition of the 3-maleimidopropionic acid (Step 3). Between every coupling, the resin is washed 3 times with *N,N*-dimethylformamide (DMF) and 3 times with isopropanol. The peptide is cleaved from the resin using 85% TFA/5% TIS/5% thioanisole and 5% phenol, followed by precipitation by dry-ice cold Et<sub>2</sub>O (Step 4). The product is purified by preparative reversed phased HPLC using a Varian (Rainin) preparative binary HPLC system: gradient elution of 30-55% B (0.045% TFA in H<sub>2</sub>O (A) and 0.045% TFA in CH<sub>3</sub>CN (B)) over 180 min at 9.5 mL/min using a Phenomenex Luna 10 μ phenyl-hexyl, 21 mm x 25 cm column and UV detector (Varian Dynamax UVD II) at λ 214 and 254 nm to afford the desired modified peptide (i.e., DAC) in >95% purity, as determined by RP-HPLC.

### Fmoc-Rink Amide MBHA Resin

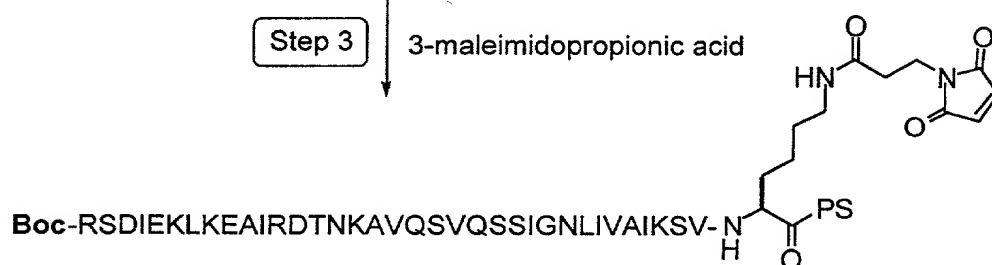
Step 1 SPPS

**Boc-RSDIEKLKEAIRDTNKAVQSVQSSIGNLIVAIKSV-Lys( Aloc)-PS**

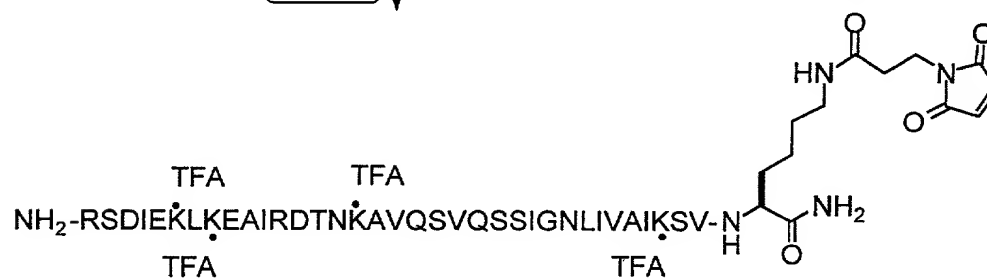
Step 2	$\text{Pd}(\text{PPh}_3)_4/\text{NMM}/\text{HOAc}/\text{CHCl}_3$
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**Boc-RSDIEKLKEAIRDTNKAVQSVQSSIGNLIVAIKSV-Lys -PS**

Step 3 3-maleimidopropionic acid



**Step 4** | 85% TFA/5% TIS/5% thioanisole/5% phenol



### Example 11

#### Preparation of a Modified anti-HPIV peptide

5 In this example, the peptide SEQ ID NO:35 is synthesized and modified to include a linker and maleimide group according to the synthesis scheme set forth below. As reported in U.S. Pat. Nos. 6,013,236 and 6,020,459, SEQ ID NO:35 inhibits viral infection of human parainfluenza virus 3 (HPIV3), including inhibiting fusion and syncytia formation between HPIV3-infected Hep2 cells and uninfected CV-1W cells.

10 Solid phase peptide synthesis of the modified peptide on a 100  $\mu$ mole scale is performed using manual solid-phase synthesis, a Symphony Peptide Synthesizer and Fmoc protected Rink Amide MBHA. The following protected amino acids are sequentially added to resin: Fmoc-Lys(Aloc)-OH, Fmoc-Leu-OH, Fmoc-  
15 Lys(Boc)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Ile-OH, Fmoc-Trp(Boc)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Leu-OH, Fmoc-Asp(tBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ala-OH, Fmoc-Lys(Boc)-OH, Fmoc-Asn(Trt)-  
20 OH, Fmoc-Leu-OH, Fmoc-Ile-OH, Fmoc-Glu(tBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Ile-OH, Fmoc-Asp(tBu)-OH, Fmoc-Ile-OH, Fmoc-Pro-OH, Fmoc-Asp(tBu)-OH, Fmoc-Leu-OH, Fmoc-Gln(Trt)-OH, Fmoc-Ala-OH, Fmoc-Val-OH, Fmoc-Ser(tBu)-OH, Fmoc-Asn(Trt)-OH. They are dissolved in *N,N*-dimethylformamide (DMF) and, according to the sequence, activated using *O*-benzotriazol-1-yl-*N,N,N',N'*-tetramethyl-uronium hexafluorophosphate (HBTU) and Diisopropylethylamine (DIEA). Removal of the Fmoc protecting group is achieved using a solution of 20% (V/V) piperidine in *N,N*-dimethylformamide (DMF) for 20 minutes (step 1). The selective deprotection of the Lys (Aloc) group is performed manually and accomplished by treating the resin with a solution of 3  
30 eq of Pd(PPh<sub>3</sub>)<sub>4</sub> dissolved in 5 mL of CHCl<sub>3</sub>:NMM:HOAc (18:1:0.5) for 2 h (Step



2). The resin is then washed with  $\text{CHCl}_3$  (6 x 5 mL), 20% HOAc in DCM (6 x 5 mL), DCM (6 x 5 mL), and DMF (6 x 5 mL). The synthesis is then re-automated for the addition of the 3-maleimidopropionic acid (Step 3). Between every coupling, the resin is washed 3 times with *N,N*-dimethylformamide (DMF) and 3  
5 times with isopropanol. The peptide is cleaved from the resin using 85% TFA/5% TIS/5% thioanisole and 5% phenol, followed by precipitation by dry-ice cold  $\text{Et}_2\text{O}$  (Step 4). The product is purified by preparative reversed phased HPLC using a Varian (Rainin) preparative binary HPLC system: gradient elution of 30-55% B (0.045% TFA in  $\text{H}_2\text{O}$  (A) and 0.045% TFA in  $\text{CH}_3\text{CN}$  (B)) over 180 min at 9.5  
10 mL/min using a Phenomenex Luna 10  $\mu$  phenyl-hexyl, 21 mm x 25 cm column and UV detector (Varian Dynamax UVD II) at  $\lambda$  214 and 254 nm to afford the desired modified peptide (i.e., DAC) in >95% purity, as determined by RP-HPLC.

005060" E5E2E50

### Fmoc-Rink Amide MBHA Resin

Step 1

**SPPS**

**Boc-NSVALDPIDISIELNKA KSDLEESKEWIRRSNQKL -Lys(Aloc)-PS**

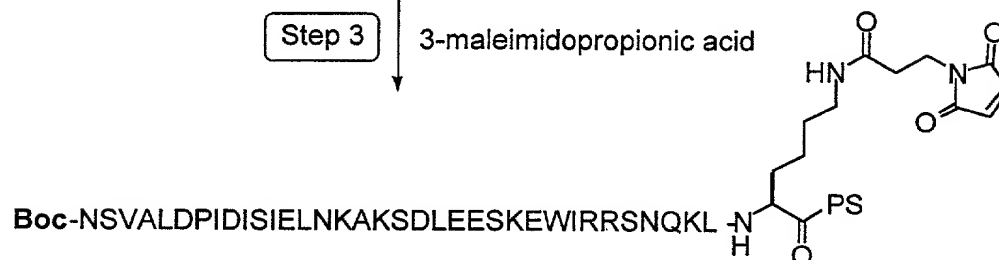
### Step 2

$\text{Pd}(\text{PPh}_3)_4/\text{NMM}/\text{HOAc}/\text{CHCl}_3$

**Boc-NSVALDPIDISIELNKA KSDLEESKEWIRRSNQKL -Lys-PS**

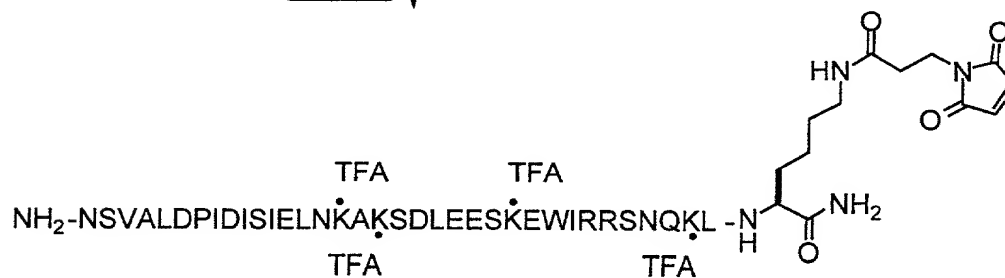
### Step 3

3-maleimidopropionic acid



### Step 4

85% TFA/5% TIS/5% thioanisole/5% phenol



## Example 12

### Preparation of a Modified anti-HPIV peptide

In this example, the peptide SEQ ID NO:38 is synthesized and modified to include a linker and maleimide group according to the synthesis scheme set forth below. As reported in U.S. Pat. Nos. 6,013,236 and 6,020,459, SEQ ID NO: 38 inhibits viral infection of human parainfluenza virus 3 (HPIV3), including inhibiting fusion and syncytia formation between HPIV3-infected Hep2 cells and uninfected CV-1W cells.

Solid phase peptide synthesis of the modified peptide analog on a 100  $\mu$ mole scale is performed using manual solid-phase synthesis, a Symphony Peptide Synthesizer and Fmoc protected Rink Amide MBHA. The following protected amino acids are sequentially added to resin: Fmoc-Ile-OH, Fmoc-Ser(tBu)-OH, Fmoc-Asp(tBu)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Ile-OH, Fmoc-Trp(Boc)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Leu-OH, Fmoc-Asp(tBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ala-OH, Fmoc-Lys(Boc)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Leu-OH, Fmoc-Glu(tBu)-OH, Fmoc-Ile-OH, Fmoc-Ser(tBu)-OH, Fmoc-Ile-OH, Fmoc-Asp(tBu)-OH, Fmoc-Ile-OH, Fmoc-Pro-OH, Fmoc-Asp(tBu)-OH, Fmoc-Leu-OH, Fmoc-Ala-OH BOC-Lys(Aloc)-OH. They are dissolved in *N,N*-dimethylformamide (DMF) and, according to the sequence, activated using *O*-benzotriazol-1-yl-*N,N,N,N*-tetramethyl-uronium hexafluorophosphate (HBTU) and Diisopropylethylamine (DIEA). Removal of the Fmoc protecting group is achieved using a solution of 20% (V/V) piperidine in *N,N*-dimethylformamide (DMF) for 20 minutes (step 1). The selective deprotection of the Lys (Aloc) group is performed manually and accomplished by treating the resin with a solution of 3 eq of  $\text{Pd}(\text{PPh}_3)_4$  dissolved in 5 mL of  $\text{CHCl}_3$ :NMM:HOAc (18:1:0.5) for 2 h (Step 2). The resin is then washed

with  $\text{CHCl}_3$  (6 x 5 mL), 20% HOAc in DCM (6 x 5 mL), DCM (6 x 5 mL), and DMF (6 x 5 mL). The synthesis is then re-automated for the addition of the 3-maleimidopropionic acid (Step 3). Between every coupling, the resin is washed 3 times with *N,N*-dimethylformamide (DMF) and 3 times with isopropanol. The peptide is cleaved from the resin using 85% TFA/5% TIS/5% thioanisole and 5% phenol, followed by precipitation by dry-ice cold  $\text{Et}_2\text{O}$  (Step 4). The product is purified by preparative reversed phased HPLC using a Varian (Rainin) preparative binary HPLC system: gradient elution of 30-55% B (0.045% TFA in  $\text{H}_2\text{O}$  (A) and 0.045% TFA in  $\text{CH}_3\text{CN}$  (B)) over 180 min at 9.5 mL/min using a Phenomenex Luna 10  $\mu$  phenyl-hexyl, 21 mm x 25 cm column and UV detector (Varian Dynamax UVD II) at  $\lambda$  214 and 254 nm to afford the desired modified peptide (i.e., DAC) in >95% purity, as determined by RP-HPLC.

Fmoc-Rink Amide MBHA Resin

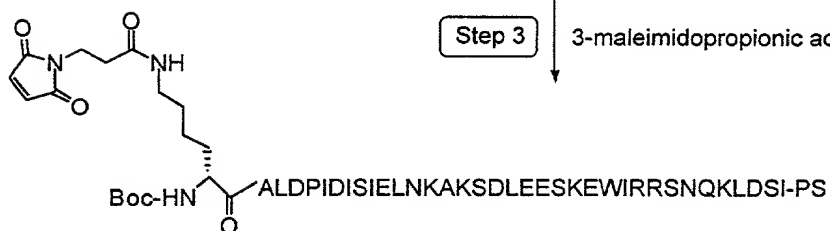
Step 1 ↓ SPPS

Boc-Lys(Aloc)-ALDPIDISIELNKA<sup>•</sup>SDLEESKEWIRRSNQKLDSI<sup>•</sup> -PS

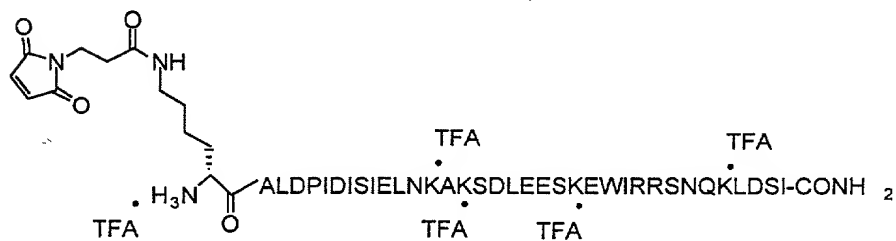
Step 2 ↓ Pd(PPh<sub>3</sub>)<sub>4</sub>/NMM/HOAc/CHCl<sub>3</sub>

Boc-Lys-ALDPIDISIELNKA<sup>•</sup>SDLEESKEWIRRSNQKLDSI<sup>•</sup>- PS

Step 3 ↓ 3-maleimidopropionic acid



Step 4 ↓ 85% TFA/5% TIS/5% thioanisole/5% phenol



005000 666666

### Example 13

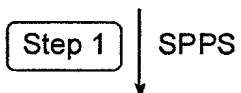
#### Preparation of a Modified anti-HPIV peptide

5           In this example, the peptide SEQ ID NO:39 is synthesized and modified to include a linker and maleimide group according to the synthesis scheme set forth below. As reported in U.S. Pat. Nos. 6,013,236 and 6,020,459, SEQ ID NO:39 inhibits viral infection of human parainfluenza virus 3 (HPIV3), including inhibiting fusion and syncytia formation between HPIV3-infected Hep2 cells and  
10           uninfected CV-1W cells.

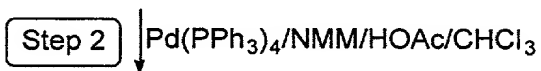
          Solid phase peptide synthesis of the modified peptide on a 100  $\mu$ mole scale is performed using manual solid-phase synthesis, a Symphony Peptide Synthesizer and Fmoc protected Rink Amide MBHA. The following protected amino acids are  
15           sequentially added to resin: Fmoc-Lys(Aloc)-OH, Fmoc-Gly-OH, Fmoc-Ile-OH, Fmoc-Ser(tBu)-OH, Fmoc-Asp(tBu)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Gly-OH, Fmoc-Asn(Trt)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Ile-OH, Fmoc-Trp(Boc)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Glu(tBu)-  
20           OH, Fmoc-Leu-OH, Fmoc-Asp(tBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ala-OH, Fmoc-Lys(Boc)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Leu-OH, Fmoc-Glu(tBu)-OH, Fmoc-Ile-OH, Fmoc-Ser(tBu)-OH, Fmoc-Ile-OH, Fmoc-Asp(tBu)-OH, Fmoc-Ile-OH, Fmoc-Pro-OH, Fmoc-Asp(tBu)-OH, Fmoc-Leu-OH. They are dissolved in *N,N*-dimethylformamide (DMF) and, according to the  
25           sequence, activated using *O*-benzotriazol-1-yl-*N,N,N',N'*-tetramethyl-uronium hexafluorophosphate (HBTU) and Diisopropylethylamine (DIEA). Removal of the Fmoc protecting group is achieved using a solution of 20% (V/V) piperidine in *N,N*-dimethylformamide (DMF) for 20 minutes (step 1). The selective deprotection of the Lys (Aloc) group is performed manually and accomplished by treating the  
30           resin with a solution of 3 eq of  $\text{Pd}(\text{PPh}_3)_4$  dissolved in 5 mL of

CHCl<sub>3</sub>:NMM:HOAc (18:1:0.5) for 2 h (Step 2). The resin is then washed with CHCl<sub>3</sub> (6 x 5 mL), 20% HOAc in DCM (6 x 5 mL), DCM (6 x 5 mL), and DMF (6 x 5 mL). The synthesis is then re-automated for the addition of the 3-maleimidopropionic acid (Step 3). Between every coupling, the resin is washed 3 times with *N,N*-dimethylformamide (DMF) and 3 times with isopropanol. The peptide is cleaved from the resin using 85% TFA/5% TIS/5% thioanisole and 5% phenol, followed by precipitation by dry-ice cold Et<sub>2</sub>O (Step 4). The product is purified by preparative reversed phased HPLC using a Varian (Rainin) preparative binary HPLC system: gradient elution of 30-55% B (0.045% TFA in H<sub>2</sub>O (A) and 0.045% TFA in CH<sub>3</sub>CN (B)) over 180 min at 9.5 mL/min using a Phenomenex Luna 10 μ phenyl-hexyl, 21 mm x 25 cm column and UV detector (Varian Dynamax UVD II) at λ 214 and 254 nm to afford the desired modified peptide (i.e., DAC) in >95% purity, as determined by RP-HPLC.

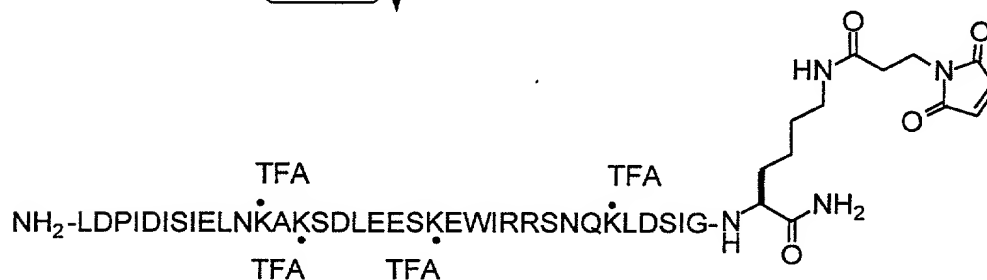
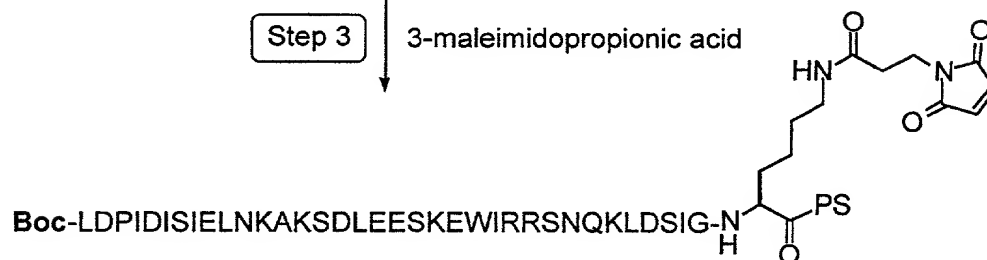
Fmoc-Rink Amide MBHA Resin



**Boc-LDPIDISIELNKAQSDLEESKEWIRRSNQKLDSIG-Lys( Aloc)-PS**



**Boc-LDPIDISIELNKAQSDLEESKEWIRRSNQKLDSIG-Lys -PS**



005060" E6E2960



#### Example 14

##### Preparation of a Modified anti-HPIV peptide

5            In this example, the peptide SEQ ID NO:40 is synthesized and modified to include a linker and maleimide group according to the synthesis scheme set forth below. As reported in U.S. Pat. Nos. 6,013,236 and 6,020,459, SEQ ID NO. inhibits viral infection of human parainfluenza virus 3 (HPIV3), including inhibiting fusion and syncytia formation between HPIV3-infected Hep2 cells and  
10           uninfected CV-1W cells.

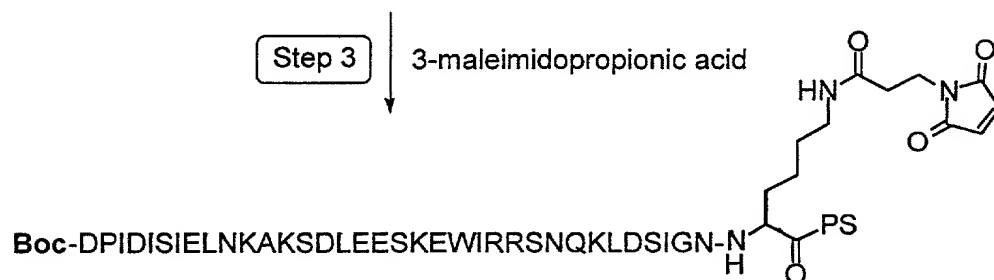
             Solid phase peptide synthesis of the modified peptide on a 100  $\mu$ mole scale is performed using manual solid-phase synthesis, a Symphony Peptide Synthesizer and Fmoc protected Rink Amide MBHA. The following protected  
15           amino acids are sequentially added to resin: Fmoc-Lys(Aloc)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Gly-OH, Fmoc-Ile-OH, Fmoc-Ser(tBu)-OH, Fmoc-Asp(tBu)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Gly-OH, Fmoc-Asn(Trt)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Ile-OH, Fmoc-Trp(Boc)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ser(tBu)-OH,  
20           Fmoc-Glu(tBu)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Leu-OH, Fmoc-Asp(tBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ala-OH, Fmoc-Lys(Boc)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Leu-OH, Fmoc-Glu(tBu)-OH, Fmoc-Ile-OH, Fmoc-Ser(tBu)-OH, Fmoc-Ile-OH, Fmoc-Asp(tBu)-OH, Fmoc-Ile-OH, Fmoc-Pro-OH, Fmoc-Asp(tBu)-OH. They are dissolved in *N,N*-dimethylformamide (DMF) and,  
25           according to the sequence, activated using *O*-benzotriazol-1-yl-*N,N,N',N'*-tetramethyl-uronium hexafluorophosphate (HBTU) and Diisopropylethylamine (DIEA). Removal of the Fmoc protecting group is achieved using a solution of 20% (V/V) piperidine in *N,N*-dimethylformamide (DMF) for 20 minutes (step 1). The selective deprotection of the Lys (Aloc) group is performed manually and  
30           accomplished by treating the resin with a solution of 3 eq of  $\text{Pd}(\text{PPh}_3)_4$  dissolved in 5 mL of  $\text{CHCl}_3$ :NMM:HOAc (18:1:0.5) for 2 h (Step 2). The resin is then washed

with  $\text{CHCl}_3$  (6 x 5 mL), 20% HOAc in DCM (6 x 5 mL), DCM (6 x 5 mL), and DMF (6 x 5 mL). The synthesis is then re-automated for the addition of the 3-maleimidopropionic acid (Step 3). Between every coupling, the resin is washed 3 times with *N,N*-dimethylformamide (DMF) and 3 times with isopropanol. The peptide is cleaved from the resin using 85% TFA/5% TIS/5% thioanisole and 5% phenol, followed by precipitation by dry-ice cold  $\text{Et}_2\text{O}$  (Step 4). The product is purified by preparative reversed phased HPLC using a Varian (Rainin) preparative binary HPLC system: gradient elution of 30-55% B (0.045% TFA in  $\text{H}_2\text{O}$  (A) and 0.045% TFA in  $\text{CH}_3\text{CN}$  (B)) over 180 min at 9.5 mL/min using a Phenomenex Luna 10  $\mu$  phenyl-hexyl, 21 mm x 25 cm column and UV detector (Varian Dynamax UVD II) at  $\lambda$  214 and 254 nm to afford the desired modified peptide (i.e., DAC) in >95% purity, as determined by RP-HPLC.

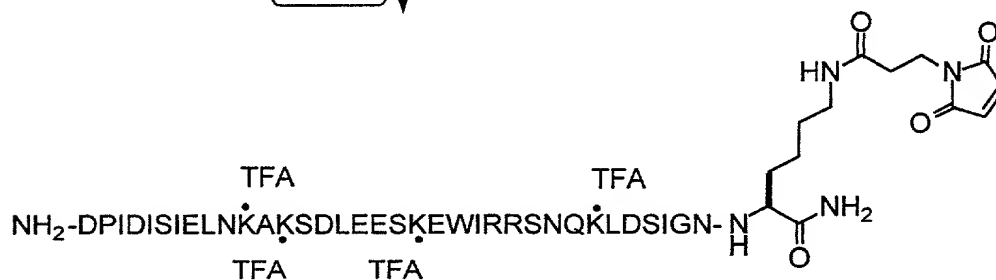
Step 1 | SPPS

Step 2 |  $\text{Pd}(\text{PPh}_3)_4/\text{NMM}/\text{HOAc}/\text{CHCl}_3$

Step 3 3-maleimidopropionic acid



**Step 4** 85% TFA/5% TIS/5% thioanisole/5% phenol



### Example 15

#### Preparation of a Modified anti-HPIV peptide

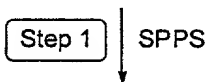
5            In this example, the peptide SEQ ID NO:41 is synthesized and modified to include a linker and maleimide group according to the synthesis scheme set forth below. As reported in U.S. Pat. Nos. 6,013,236 and 6,020,459, SEQ ID NO:41 inhibits viral infection of human parainfluenza virus 3 (HPIV3), including inhibiting fusion and syncytia formation between HPIV3-infected Hep2 cells and  
10            uninfected CV-1W cells.

             Solid phase peptide synthesis of the modified peptide on a 100  $\mu$ mole scale is performed using manual solid-phase synthesis, a Symphony Peptide Synthesizer and Fmoc protected Rink Amide MBHA. The following protected  
15            amino acids are sequentially added to resin: Fmoc-Trp(Boc)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Gly-OH, Fmoc-Ile-OH, Fmoc-Ser(tBu)-OH, Fmoc-Asp(tBu)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Gly-OH, Fmoc-Asn(Trt)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Ile-OH, Fmoc-Trp(Boc)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ser(tBu)-OH,  
20            Fmoc-Glu(tBu)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Leu-OH, Fmoc-Asp(tBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ala-OH, Fmoc-Lys(Boc)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Leu-OH, Fmoc-Glu(tBu)-OH, Fmoc-Ile-OH, Fmoc-Ser(tBu)-OH, Fmoc-Ile-OH, Fmoc-Asp(tBu)-OH, Fmoc-Ile-OH, Fmoc-Pro-OH Boc-Lys(Aloc)-OH,. They are dissolved in *N,N*-dimethylformamide (DMF) and,  
25            according to the sequence, activated using *O*-benzotriazol-1-yl-*N,N,N,N*-tetramethyl-uronium hexafluorophosphate (HBTU) and Diisopropylethylamine (DIEA). Removal of the Fmoc protecting group is achieved using a solution of 20% (V/V) piperidine in *N,N*-dimethylformamide (DMF) for 20 minutes (step 1). The selective deprotection of the Lys (Aloc) group is performed manually and  
30            accomplished by treating the resin with a solution of 3 eq of Pd(PPh<sub>3</sub>)<sub>4</sub> dissolved in 5 mL of CHCl<sub>3</sub>:NMM:HOAc (18:1:0.5) for 2 h (Step 2). The resin is then washed

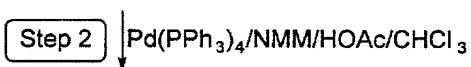
with  $\text{CHCl}_3$  (6 x 5 mL), 20% HOAc in DCM (6 x 5 mL), DCM (6 x 5 mL), and DMF (6 x 5 mL). The synthesis is then re-automated for the addition of the 3-maleimidopropionic acid (Step 3). Between every coupling, the resin is washed 3 times with *N,N*-dimethylformamide (DMF) and 3 times with isopropanol. The peptide is cleaved from the resin using 85% TFA/5% TIS/5% thioanisole and 5% phenol, followed by precipitation by dry-ice cold  $\text{Et}_2\text{O}$  (Step 4). The product is purified by preparative reversed phased HPLC using a Varian (Rainin) preparative binary HPLC system: gradient elution of 30-55% B (0.045% TFA in  $\text{H}_2\text{O}$  (A) and 0.045% TFA in  $\text{CH}_3\text{CN}$  (B)) over 180 min at 9.5 mL/min using a Phenomenex Luna 10  $\mu$  phenyl-hexyl, 21 mm x 25 cm column and UV detector (Varian Dynamax UVD II) at  $\lambda$  214 and 254 nm to afford the desired modified peptide (i.e., DAC) in >95% purity, as determined by RP-HPLC.

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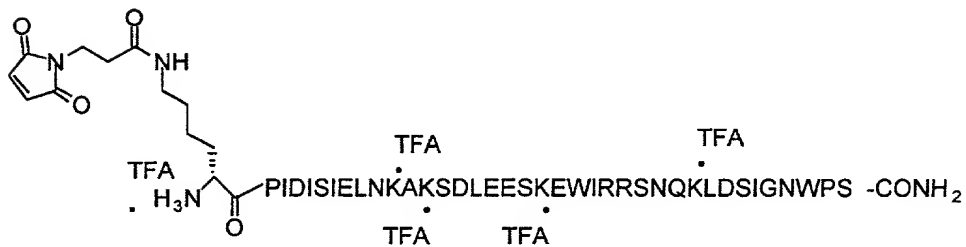
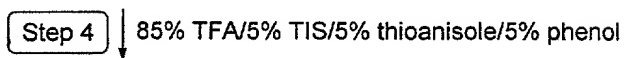
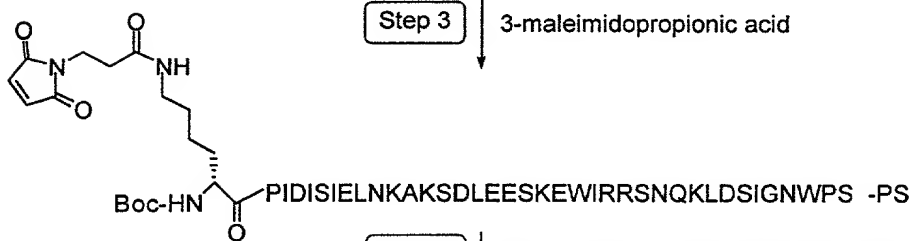
Fmoc-Rink Amide MBHA Resin



Boc-Lys(Aloc)-PIDISIELNKA<sup>•</sup>AKSDLEESKEWIRRSNQKLDSIGNW-PS



Boc-Lys-PIDISIELNKA<sup>•</sup>AKSDLEESKEWIRRSNQKLDSIGNWPS



005060" E5E2960

### Example 16

#### Preparation of a Modified anti-HPIV peptide

5            In this example, the peptide SEQ ID NO:42 is synthesized and modified to include a linker and maleimide group according to the synthesis scheme set forth below. As reported in U.S. Pat. Nos. 6,013,236 and 6,020,459, SEQ ID NO:42 inhibits viral infection of human parainfluenza virus 3 (HPIV3), including inhibiting fusion and syncytia formation between HPIV3-infected Hep2 cells and  
10           uninfected CV-1W cells.

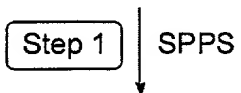
             Solid phase peptide synthesis of the modified peptide on a 100  $\mu$ mole scale is performed using manual solid-phase synthesis, a Symphony Peptide Synthesizer and Fmoc protected Rink Amide MBHA. The following protected amino acids are  
15           sequentially added to resin: Fmoc-Lys(Aloc)-OH, Fmoc-His(Boc)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Gly-OH, Fmoc-Ile-OH, Fmoc-Ser(tBu)-OH, Fmoc-Asp(tBu)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Gly-OH, Fmoc-Asn(Trt)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Ile-OH, Fmoc-Trp(Boc)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Leu-OH, Fmoc-Asp(tBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ala-OH, Fmoc-Lys(Boc)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Leu-OH, Fmoc-Glu(tBu)-OH, Fmoc-Ile-OH, Fmoc-Ser(tBu)-OH, Fmoc-Ile-OH, Fmoc-Asp(tBu)-OH, Fmoc-Ile-OH. They are dissolved in *N,N*-dimethylformamide (DMF) and,  
25           according to the sequence, activated using *O*-benzotriazol-1-yl-*N,N,N',N'*-tetramethyl-uronium hexafluorophosphate (HBTU) and Diisopropylethylamine (DIEA). Removal of the Fmoc protecting group is achieved using a solution of 20% (V/V) piperidine in *N,N*-dimethylformamide (DMF) for 20 minutes (step 1). The selective deprotection of the Lys (Aloc) group is performed manually and  
30           accomplished by treating the resin with a solution of 3 eq of Pd(PPh<sub>3</sub>)<sub>4</sub> dissolved in

5 mL of  $\text{CHCl}_3$ :NMM:HOAc (18:1:0.5) for 2 h (Step 2). The resin is then washed with  $\text{CHCl}_3$  (6 x 5 mL), 20% HOAc in DCM (6 x 5 mL), DCM (6 x 5 mL), and DMF (6 x 5 mL). The synthesis is then re-automated for the addition of the 3-maleimidopropionic acid (Step 3). Between every coupling, the resin is washed 3 times with *N,N*-dimethylformamide (DMF) and 3 times with isopropanol. The peptide is cleaved from the resin using 85% TFA/5% TIS/5% thioanisole and 5% phenol, followed by precipitation by dry-ice cold  $\text{Et}_2\text{O}$  (Step 4). The product is purified by preparative reversed phased HPLC using a Varian (Rainin) preparative binary HPLC system: gradient elution of 30-55% B (0.045% TFA in  $\text{H}_2\text{O}$  (A) and 0.045% TFA in  $\text{CH}_3\text{CN}$  (B)) over 180 min at 9.5 mL/min using a Phenomenex Luna 10  $\mu$  phenyl-hexyl, 21 mm x 25 cm column and UV detector (Varian Dynamax UVD II) at  $\lambda$  214 and 254 nm to afford the desired modified peptide (i.e., DAC) in >95% purity, as determined by RP-HPLC

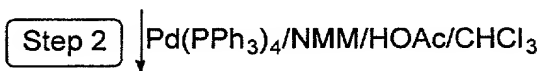
005060" FEE2960



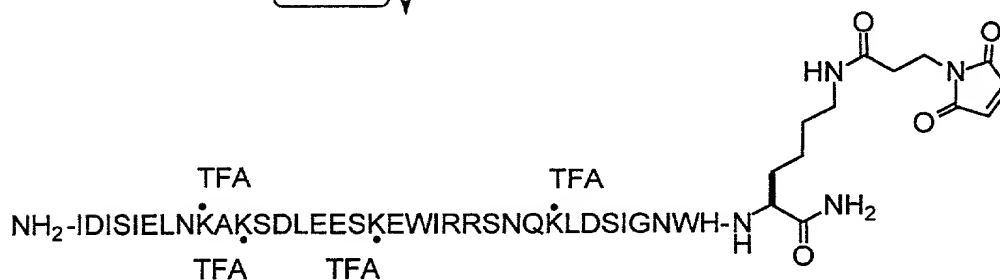
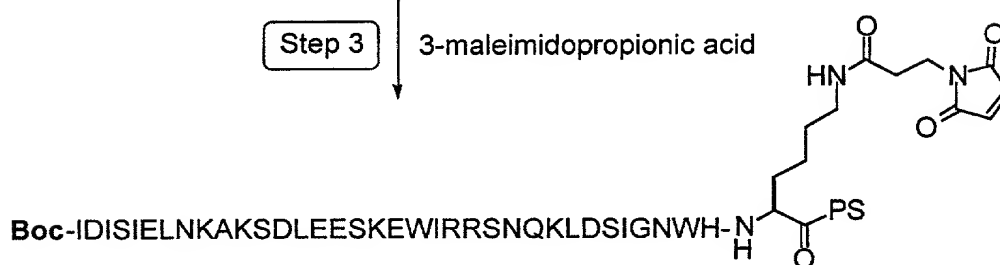
Fmoc-Rink Amide MBHA Resin



**Boc-IDISIELNKAQSDLEESKEWIRRSNQKLDSIGNWH-Lys(Alloc)-PS**



**Boc-IDISIELNKAQSDLEESKEWIRRSNQKLDSIGNWH-Lys-PS**



005060" EEEEEE

### Example 17

#### Preparation of a Modified anti-MeV peptide

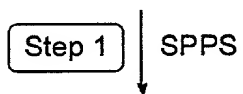
5            In this example, the peptide SEQ ID NO:77. is synthesized and modified to include a linker and maleimide group according to the synthesis scheme set forth below. As reported in U.S. Pat. Nos. 6,013,236 and 6,020,459, SEQ ID NO:77 inhibits viral infection of measles virus (MeV), including inhibiting fusion and syncytia formation between MeV-infected and uninfected Vero cells.

10

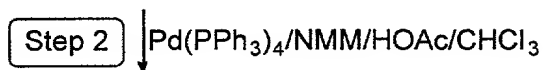
          Solid phase peptide synthesis of the modified peptide on a 100  $\mu$ mole scale is performed using manual solid-phase synthesis, a Symphony Peptide Synthesizer and Fmoc protected Rink Amide MBHA. The following protected amino acids are  
15 sequentially added to resin: Fmoc-Lys(Aloc)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Leu-OH, Fmoc-Leu-OH, Fmoc-Glu(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ala-OH, Fmoc-Glu(tBu)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ala-OH, Fmoc-Ile-OH, Fmoc-Ala-OH, Fmoc-Asn(Trt)-OH, Fmoc-Gly-OH, Fmoc-Leu-OH, Fmoc-Asn(Trt)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Gly-OH, Fmoc-Val-OH, Fmoc-  
20 Asp(tBu)-OH, Fmoc-Leu-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Leu-OH, Fmoc-Ser(tBu)-OH, Fmoc-Ile-OH, Fmoc-Pro-OH, Fmoc-Pro-OH, Fmoc-Gly-OH, Fmoc-Leu-OH, Fmoc-Asp(tBu)-OH, Fmoc-Ile-OH, Fmoc-Arg(Pbf)-OH, Fmoc-His(Boc)-OH. They are dissolved in *N,N*-dimethylformamide (DMF) and, according to the sequence, activated using *O*-benzotriazol-1-yl-*N,N,N,N*-tetramethyl-uronium hexafluorophosphate (HBTU) and Diisopropylethylamine (DIEA). Removal of the Fmoc protecting group is achieved using a solution of  
25 20% (V/V) piperidine in *N,N*-dimethylformamide (DMF) for 20 minutes (step 1). The selective deprotection of the Lys (Aloc) group is performed manually and accomplished by treating the resin with a solution of 3 eq of Pd(PPh<sub>3</sub>)<sub>4</sub> dissolved in  
30 5 mL of CHCl<sub>3</sub>:NMM:HOAc (18:1:0.5) for 2 h (Step 2). The resin is then washed with CHCl<sub>3</sub> (6 x 5 mL), 20% HOAc in DCM (6 x 5 mL), DCM (6 x 5 mL), and

DMF (6 x 5 mL). The synthesis is then re-automated for the addition of the 3-maleimidopropionic acid (Step 3). Between every coupling, the resin is washed 3 times with *N,N*-dimethylformamide (DMF) and 3 times with isopropanol. The peptide is cleaved from the resin using 85% TFA/5% TIS/5% thioanisole and 5% phenol, followed by precipitation by dry-ice cold Et<sub>2</sub>O (Step 4). The product is purified by preparative reversed phased HPLC using a Varian (Rainin) preparative binary HPLC system: gradient elution of 30-55% B (0.045% TFA in H<sub>2</sub>O (A) and 0.045% TFA in CH<sub>3</sub>CN (B)) over 180 min at 9.5 mL/min using a Phenomenex Luna 10 μ phenyl-hexyl, 21 mm x 25 cm column and UV detector (Varian Dynamax UVD II) at λ 214 and 254 nm to afford the desired modified peptide (i.e., DAC) in >95% purity, as determined by RP-HPLC.

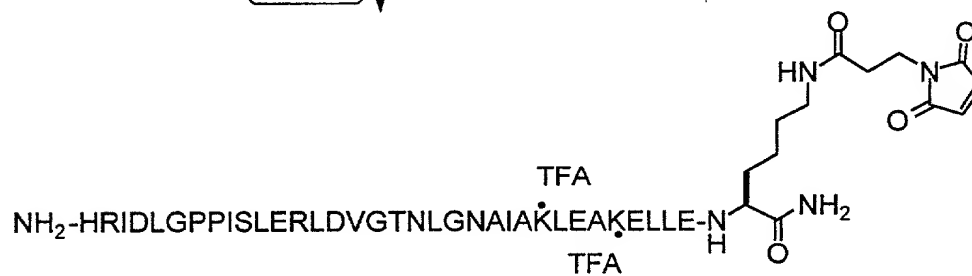
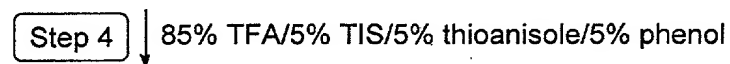
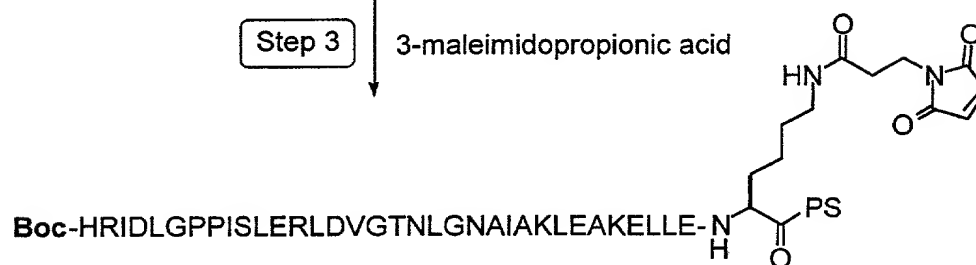
Fmoc-Rink Amide MBHA Resin



**Boc**-HRIDLGPPISLERLDVGTNLGNAIAKLEAKELLE-Lys( **Aloc**)-PS



**Boc**-HRIDLGPPISLERLDVGTNLGNAIAKLEAKELLE-Lys-PS



005050 6662960

### Example 18

#### Preparation of a Modified anti-MeV peptide

5 In this example, the peptide SEQ ID NO:79 is synthesized and modified to include a linker and maleimide group according to the synthesis scheme set forth below. As reported in U.S. Pat. Nos. 6,013,236 and 6,020,459, SEQ ID NO:79 inhibits viral infection of measles virus (MeV), including inhibiting fusion and syncytia formation between MeV-infected and uninfected Vero cells.

10 Solid phase peptide synthesis of the modified peptide on a 100  $\mu$ mole scale is performed using manual solid-phase synthesis, a Symphony Peptide Synthesizer and Fmoc protected Rink Amide MBHA. The following protected amino acids are sequentially added to resin: Fmoc-Lys(Aloc)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Leu-OH, Fmoc-Leu-OH, Fmoc-Glu(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ala-OH, Fmoc-Glu(tBu)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ala-OH, Fmoc-Ile-OH, Fmoc-Ala-OH, Fmoc-Asn(Trt)-OH, Fmoc-Gly-OH, Fmoc-Leu-OH, Fmoc-Asn(Trt)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Gly-OH, Fmoc-Val-OH, Fmoc-Asp(tBu)-OH, Fmoc-Leu-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Leu-OH, Fmoc-Ser(tBu)-OH, Fmoc-Ile-OH, Fmoc-Pro-OH, Fmoc-Pro-OH, Fmoc-Gly-OH, Fmoc-Leu-OH, Fmoc-Asp(tBu)-OH, Fmoc-Ile-OH, They are dissolved in *N,N*-dimethylformamide (DMF) and, according to the sequence, activated using *O*-benzotriazol-1-yl-*N,N,N',N'*-tetramethyl-uronium hexafluorophosphate (HBTU) and Diisopropylethylamine (DIEA). Removal of the Fmoc protecting group is achieved using a solution of 20% (V/V) piperidine in *N,N*-dimethylformamide (DMF) for 20 minutes (step 1). The selective deprotection of the Lys (Aloc) group is performed manually and accomplished by treating the resin with a solution of 3 eq of Pd(PPh<sub>3</sub>)<sub>4</sub> dissolved in 5 mL of CHCl<sub>3</sub>:NMM:HOAc (18:1:0.5) for 2 h (Step 2).

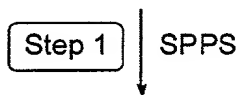
20 OH, Fmoc-Arg(Pbf)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Leu-OH, Fmoc-Ser(tBu)-OH, Fmoc-Ile-OH, Fmoc-Pro-OH, Fmoc-Pro-OH, Fmoc-Gly-OH, Fmoc-Leu-OH, Fmoc-Asp(tBu)-OH, Fmoc-Ile-OH, They are dissolved in *N,N*-dimethylformamide (DMF) and, according to the sequence, activated using *O*-benzotriazol-1-yl-*N,N,N',N'*-tetramethyl-uronium hexafluorophosphate (HBTU) and Diisopropylethylamine (DIEA). Removal of the Fmoc protecting group is achieved using a solution of 20% (V/V) piperidine in *N,N*-dimethylformamide (DMF) for 20 minutes (step 1). The selective deprotection of the Lys (Aloc) group is performed manually and accomplished by treating the resin with a solution of 3 eq of Pd(PPh<sub>3</sub>)<sub>4</sub> dissolved in 5 mL of CHCl<sub>3</sub>:NMM:HOAc (18:1:0.5) for 2 h (Step 2).

25 Diisopropylethylamine (DIEA). Removal of the Fmoc protecting group is achieved using a solution of 20% (V/V) piperidine in *N,N*-dimethylformamide (DMF) for 20 minutes (step 1). The selective deprotection of the Lys (Aloc) group is performed manually and accomplished by treating the resin with a solution of 3 eq of Pd(PPh<sub>3</sub>)<sub>4</sub> dissolved in 5 mL of CHCl<sub>3</sub>:NMM:HOAc (18:1:0.5) for 2 h (Step 2).

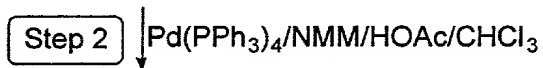
30 The resin is then washed with CHCl<sub>3</sub> (6 x 5 mL), 20% HOAc in DCM (6 x 5 mL),

DCM (6 x 5 mL), and DMF (6 x 5 mL). The synthesis is then re-automated for the addition of the 3-maleimidopropionic acid (Step 3). Between every coupling, the resin is washed 3 times with *N,N*-dimethylformamide (DMF) and 3 times with isopropanol. The peptide is cleaved from the resin using 85% TFA/5% TIS/5% thioanisole and 5% phenol, followed by precipitation by dry-ice cold Et<sub>2</sub>O (Step 4). The product is purified by preparative reversed phased HPLC using a Varian (Rainin) preparative binary HPLC system: gradient elution of 30-55% B (0.045% TFA in H<sub>2</sub>O (A) and 0.045% TFA in CH<sub>3</sub>CN (B)) over 180 min at 9.5 mL/min using a Phenomenex Luna 10 μ phenyl-hexyl, 21 mm x 25 cm column and UV detector (Varian Dynamax UVD II) at λ 214 and 254 nm to afford the desired modified peptide (i.e., DAC) in >95% purity, as determined by RP-HPLC

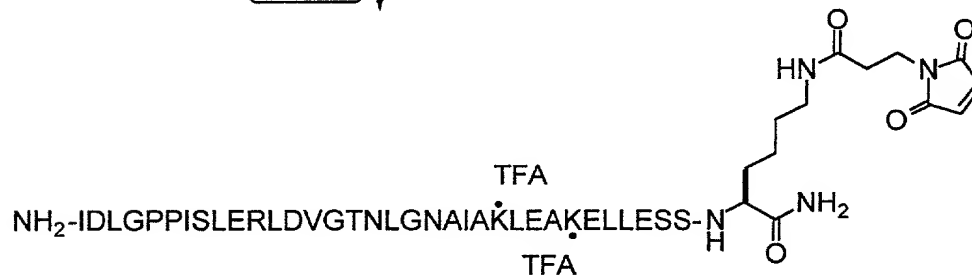
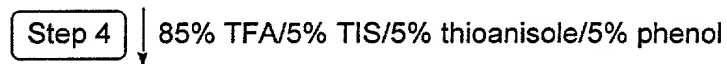
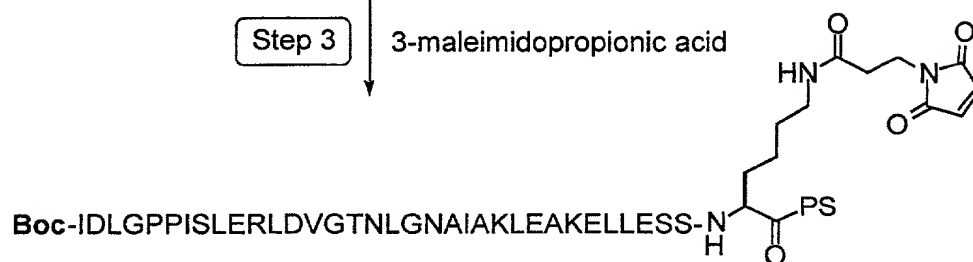
Fmoc-Rink Amide MBHA Resin



**Boc-IDLGPPISLERLDVGTNLGNIAKLEAKELLESS-Lys(Alloc)-PS**



**Boc-IDLGPPISLERLDVGTNLGNIAKLEAKELLESS-Lys-PS**



005060" EEE2360

### Example 19

#### Preparation of a Modified anti-MeV peptide

5           In this example, the peptide SEQ ID NO:81 is synthesized and modified to include a linker and maleimide group according to the synthesis scheme set forth below. As reported in U.S. Pat. Nos. 6,013,236 and 6,020,459, SEQ ID NO: 79 inhibits viral infection of measles virus (MeV), including inhibiting fusion and syncytia formation between MeV-infected and uninfected Vero cells.

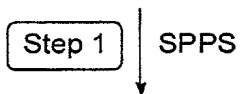
10           Solid phase peptide synthesis of the modified peptide on a 100  $\mu$ mole scale is performed using manual solid-phase synthesis, a Symphony Peptide Synthesizer and Fmoc protected Rink Amide MBHA. The following protected amino acids are sequentially added to resin: Fmoc-Lys(Aloc)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Asp(tBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Leu-OH, Fmoc-Leu-OH, Fmoc-Glu(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ala-OH, Fmoc-Glu(tBu)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ala-OH, Fmoc-Ile-OH, Fmoc-Ala-OH, Fmoc-Asn(Trt)-OH, Fmoc-Gly-OH, Fmoc-Leu-OH, Fmoc-Asn(Trt)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Gly-OH, Fmoc-Val-OH, Fmoc-Asp(tBu)-OH, Fmoc-Leu-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Leu-OH, Fmoc-Ser(tBu)-OH, Fmoc-Ile-OH, Fmoc-Pro-OH, Fmoc-Pro-OH, Fmoc-Gly-OH, Fmoc-Leu-OH. They are dissolved in *N,N*-dimethylformamide (DMF) and, according to the sequence, activated using *O*-benzotriazol-1-yl-*N,N,N',N'*-tetramethyl-uronium hexafluorophosphate (HBTU) and Diisopropylethylamine (DIEA). Removal of the Fmoc protecting group is achieved using a solution of 20% (V/V) piperidine in *N,N*-dimethylformamide (DMF) for 20 minutes (step 1). The selective deprotection of the Lys (Aloc) group is performed manually and accomplished by treating the resin with a solution of 3 eq of Pd(PPh<sub>3</sub>)<sub>4</sub> dissolved in 5 mL of CHCl<sub>3</sub>:NMM:HOAc (18:1:0.5) for 2 h (Step 2).

30           The resin is then washed with CHCl<sub>3</sub> (6 x 5 mL), 20% HOAc in DCM (6 x 5 mL), DCM (6 x 5 mL), and DMF (6 x 5 mL). The synthesis is then re-automated for the

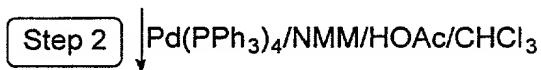


addition of the 3-maleimidopropionic acid (Step 3). Between every coupling, the resin is washed 3 times with *N,N*-dimethylformamide (DMF) and 3 times with isopropanol. The peptide is cleaved from the resin using 85% TFA/5% TIS/5% thioanisole and 5% phenol, followed by precipitation by dry-ice cold Et<sub>2</sub>O (Step 4). The product is purified by preparative reversed phased HPLC using a Varian (Rainin) preparative binary HPLC system: gradient elution of 30-55% B (0.045% TFA in H<sub>2</sub>O (A) and 0.045% TFA in CH<sub>3</sub>CN (B)) over 180 min at 9.5 mL/min using a Phenomenex Luna 10 μ phenyl-hexyl, 21 mm x 25 cm column and UV detector (Varian Dynamax UVD II) at λ 214 and 254 nm to afford the desired modified peptide (i.e., DAC) in >95% purity, as determined by RP-HPLC.

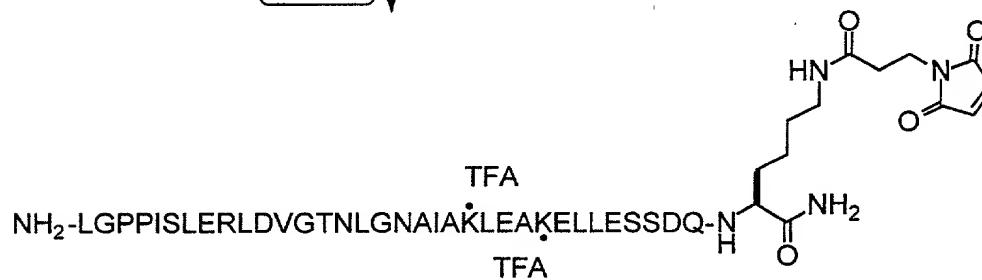
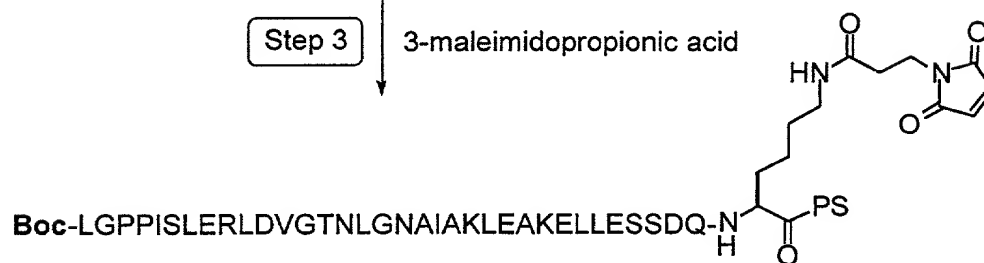
Fmoc-Rink Amide MBHA Resin



**Boc-LGPPISLERLDVGTNLGNIAKLEAKELLESSDQ-Lys(Alloc)-PS**



**Boc-LGPPISLERLDVGTNLGNIAKLEAKELLESSDQ-Lys-PS**



005060" 6362950

## Example 20

### Preparation of a Modified anti-MeV peptide

5            In this example, the peptide SEQ ID NO:84 is synthesized and modified to include a linker and maleimide group according to the synthesis scheme set forth below. As reported in U.S. Pat. Nos. 6,013,236 and 6,020,459, SEQ ID NO:84 inhibits viral infection of measles virus (MeV), including inhibiting fusion and syncytia formation between MeV-infected and uninfected Vero cells.

10            Solid phase peptide synthesis of the modified peptide on a 100  $\mu$ mole scale is performed using manual solid-phase synthesis, a Symphony Peptide Synthesizer and Fmoc protected Rink Amide MBHA. The following protected amino acids are sequentially added to resin: Fmoc-Lys(Aloc)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Leu-  
15            OH, Fmoc-Ile-OH, Fmoc-Gln(Trt)-OH, Fmoc-Asp(tBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Leu-OH, Fmoc-Leu-OH, Fmoc-Glu(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ala-OH, Fmoc-Glu(tBu)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ala-OH, Fmoc-Ile-OH, Fmoc-Ala-OH, Fmoc-Asn(Trt)-OH, Fmoc-Gly-OH, Fmoc-Leu-OH, Fmoc-Asn(Trt)-OH, Fmoc-  
20            Thr(tBu)-OH, Fmoc-Gly-OH, Fmoc-Val-OH, Fmoc-Asp(tBu)-OH, Fmoc-Leu-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Leu-OH, Fmoc-Ser(tBu)-OH, Fmoc-Ile-OH, Fmoc-Pro-OH. They are dissolved in *N,N*-dimethylformamide (DMF) and, according to the sequence, activated using *O*-benzotriazol-1-yl-*N,N,N',N'*-tetramethyl-uronium hexafluorophosphate (HBTU) and  
25            Diisopropylethylamine (DIEA). Removal of the Fmoc protecting group is achieved using a solution of 20% (V/V) piperidine in *N,N*-dimethylformamide (DMF) for 20 minutes (step 1). The selective deprotection of the Lys (Aloc) group is performed manually and accomplished by treating the resin with a solution of 3 eq of Pd(PPh<sub>3</sub>)<sub>4</sub> dissolved in 5 mL of CHCl<sub>3</sub>:NMM:HOAc (18:1:0.5) for 2 h (Step 2).  
30            The resin is then washed with CHCl<sub>3</sub> (6 x 5 mL), 20% HOAc in DCM (6 x 5 mL), DCM (6 x 5 mL), and DMF (6 x 5 mL). The synthesis is then re-automated for the

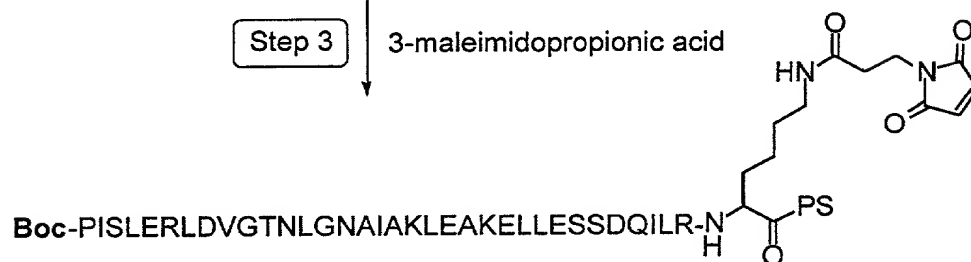
addition of the 3-maleimidopropionic acid (Step 3). Between every coupling, the resin is washed 3 times with *N,N*-dimethylformamide (DMF) and 3 times with isopropanol. The peptide is cleaved from the resin using 85% TFA/5% TIS/5% thioanisole and 5% phenol, followed by precipitation by dry-ice cold Et<sub>2</sub>O (Step 4). The product is purified by preparative reversed phased HPLC using a Varian (Rainin) preparative binary HPLC system: gradient elution of 30-55% B (0.045% TFA in H<sub>2</sub>O (A) and 0.045% TFA in CH<sub>3</sub>CN (B)) over 180 min at 9.5 mL/min using a Phenomenex Luna 10 μ phenyl-hexyl, 21 mm x 25 cm column and UV detector (Varian Dynamax UVD II) at λ 214 and 254 nm to afford the desired modified peptide (i.e., DAC) in >95% purity, as determined by RP-HPLC.

Step 1 SPPS

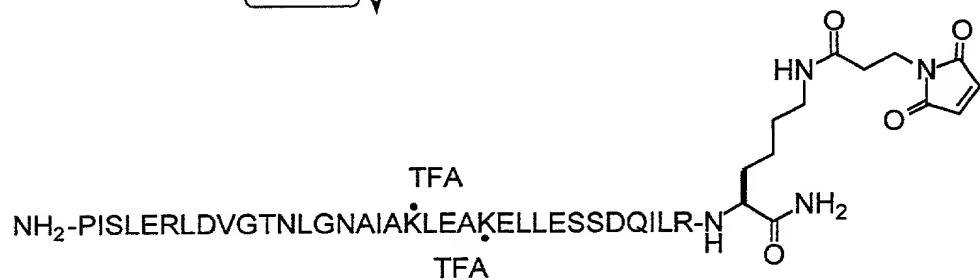
Step 2  $\downarrow$  Pd(PPh<sub>3</sub>)<sub>4</sub>/NMM/HOAc/CHCl<sub>3</sub>

Step 3

3-maleimidopropionic acid



**Step 4** ↓ 85% TFA/5% TIS/5% thioanisole/5% phenol



### Example 21

#### Preparation of a Modified anti-SIV peptide

5            In this example, the peptide SEQ ID NO:64 is synthesized and modified to include a linker and maleimide group according to the synthesis scheme set forth below. As reported in U.S. Pat. Nos. 6,013,236 and 6,020,459, SEQ ID NO:64. exhibits potent antiviral activity as a crude peptide against simian immunodeficiency virus (SIV).

10            Solid phase peptide synthesis of the modified peptide on a 100  $\mu$ mole scale is performed using manual solid-phase synthesis, a Symphony Peptide Synthesizer and Fmoc protected Rink Amide MBHA. The following protected amino acids are sequentially added to resin: Fmoc-Lys(Aloc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Leu-OH, Fmoc-Glu(tBu)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Met-OH, Fmoc-Asn(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Ile-OH, Fmoc-Gln(Trt)-OH, Fmoc-Ala-OH, Fmoc-Glu(tBu)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Leu-OH, Fmoc-Leu-OH, Fmoc-Ala-OH, Fmoc-Thr(tBu)-OH, Fmoc-Ile-OH, Fmoc-Asn(Trt)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Leu-OH, Fmoc-Phe-OH, Fmoc-Asp(tBu)-OH, Fmoc-Val-OH, Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Trp(Boc)-OH. They are dissolved in *N,N*-dimethylformamide (DMF) and, according to the sequence, activated using *O*-benzotriazol-1-yl-*N,N,N,N*-tetramethyl-uronium hexafluorophosphate (HBTU) and Diisopropylethylamine (DIEA). Removal of the Fmoc protecting group is achieved using a solution of 20% (V/V) piperidine in *N,N*-dimethylformamide (DMF) for 20 minutes (step 1). The selective deprotection of the Lys (Aloc) group is performed manually and accomplished by treating the resin with a solution of 3 eq of Pd(PPh<sub>3</sub>)<sub>4</sub> dissolved in 5 mL of CHCl<sub>3</sub>:NMM:HOAc (18:1:0.5) for 2 h (Step 2). The resin is then washed

15

20

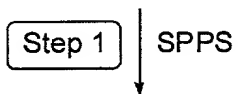
25

30

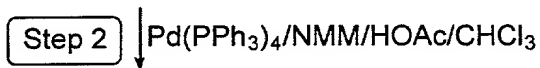
with  $\text{CHCl}_3$  (6 x 5 mL), 20% HOAc in DCM (6 x 5 mL), DCM (6 x 5 mL), and DMF (6 x 5 mL). The synthesis is then re-automated for the addition of the 3-maleimidopropionic acid (Step 3). Between every coupling, the resin is washed 3 times with *N,N*-dimethylformamide (DMF) and 3 times with isopropanol. The peptide is cleaved from the resin using 85% TFA/5% TIS/5% thioanisole and 5% phenol, followed by precipitation by dry-ice cold  $\text{Et}_2\text{O}$  (Step 4). The product is purified by preparative reversed phased HPLC using a Varian (Rainin) preparative binary HPLC system: gradient elution of 30-55% B (0.045% TFA in  $\text{H}_2\text{O}$  (A) and 0.045% TFA in  $\text{CH}_3\text{CN}$  (B)) over 180 min at 9.5 mL/min using a Phenomenex Luna 10  $\mu$  phenyl-hexyl, 21 mm x 25 cm column and UV detector (Varian Dynamax UVD II) at  $\lambda$  214 and 254 nm to afford the desired modified peptide (i.e., DAC) in >95% purity, as determined by RP-HPLC.

005060" EEE2960

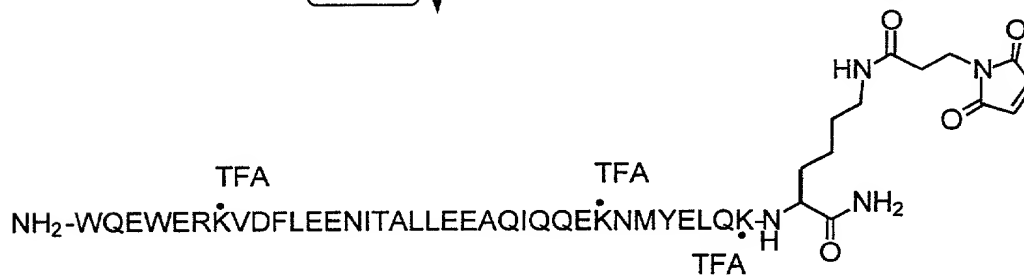
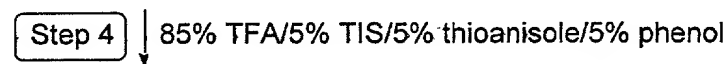
Fmoc-Rink Amide MBHA Resin



Boc-WQEWERKVDLFLEENITALLEEAIQQEKNMYELQK-Lys(Aloc)-PS



Boc-WQEWERKVDLFLEENITALLEEAIQQEKNMYELQK-Lys-PS



005060" 6662360



## Example 22

### Preparation of a Modified anti-SIV peptide

5            In this example, the peptide SEQ ID NO:65 is synthesized and modified to include a linker and maleimide group according to the synthesis scheme set forth below. As reported in U.S. Pat. Nos. 6,013,236 and 6,020,459, SEQ ID NO:65 exhibits potent antiviral activity as a crude peptide against simian immunodeficiency virus (SIV).

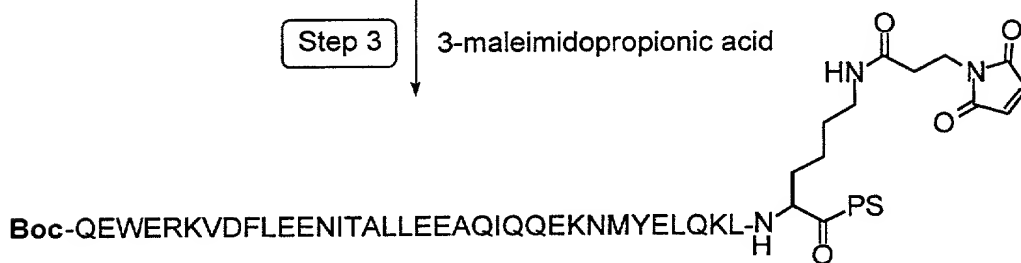
10            Solid phase peptide synthesis of the modified peptide on a 100  $\mu$ mole scale is performed using manual solid-phase synthesis, a Symphony Peptide Synthesizer and Fmoc protected Rink Amide MBHA. The following protected amino acids are sequentially added to resin: Fmoc-Lys(Aloc)-OH, Fmoc-Leu-OH, Fmoc-  
15            Lys(Boc)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Leu-OH, Fmoc-Glu(tBu)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Met-OH, Fmoc-Asn(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Ile-OH, Fmoc-Gln(Trt)-OH, Fmoc-Ala-OH, Fmoc-Glu(tBu)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Leu-OH, Fmoc-Leu-OH, Fmoc-Ala-OH, Fmoc-Thr(tBu)-OH, Fmoc-Ile-OH,  
20            Fmoc-Asn(Trt)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Leu-OH, Fmoc-Phe-OH, Fmoc-Asp(tBu)-OH, Fmoc-Val-OH, Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Gln(Trt)-OH. They are dissolved in *N,N*-dimethylformamide (DMF) and, according to the sequence, activated using *O*-benzotriazol-1-yl-*N,N,N,N*-tetramethyl-uronium hexafluorophosphate (HBTU) and Diisopropylethylamine (DIEA). Removal of the Fmoc protecting group is achieved using a solution of  
25            20% (V/V) piperidine in *N,N*-dimethylformamide (DMF) for 20 minutes (step 1). The selective deprotection of the Lys (Aloc) group is performed manually and accomplished by treating the resin with a solution of 3 eq of Pd(PPh<sub>3</sub>)<sub>4</sub> dissolved in  
30            5 mL of CHCl<sub>3</sub>:NMM:HOAc (18:1:0.5) for 2 h (Step 2). The resin is then washed with CHCl<sub>3</sub> (6 x 5 mL), 20% HOAc in DCM (6 x 5 mL), DCM (6 x 5 mL), and

DMF (6 x 5 mL). The synthesis is then re-automated for the addition of the 3-maleimidopropionic acid (Step 3). Between every coupling, the resin is washed 3 times with *N,N*-dimethylformamide (DMF) and 3 times with isopropanol. The peptide is cleaved from the resin using 85% TFA/5% TIS/5% thioanisole and 5% phenol, followed by precipitation by dry-ice cold Et<sub>2</sub>O (Step 4). The product is purified by preparative reversed phased HPLC using a Varian (Rainin) preparative binary HPLC system: gradient elution of 30-55% B (0.045% TFA in H<sub>2</sub>O (A) and 0.045% TFA in CH<sub>3</sub>CN (B)) over 180 min at 9.5 mL/min using a Phenomenex Luna 10 μ phenyl-hexyl, 21 mm x 25 cm column and UV detector (Varian Dynamax UVD II) at λ 214 and 254 nm to afford the desired modified peptide (i.e., DAC) in >95% purity, as determined by RP-HPLC.

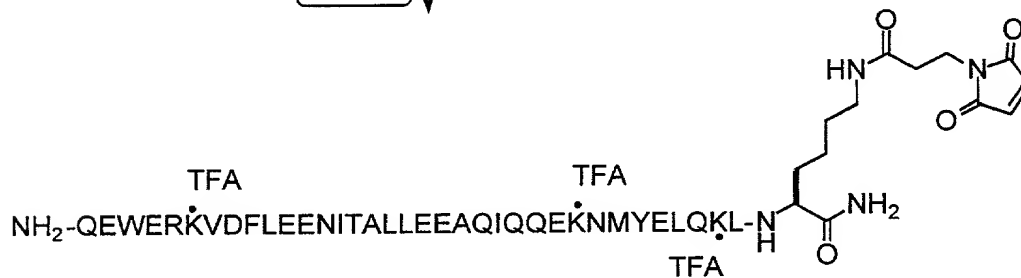
Step 1 ↓ SPSS

Step 2  $\downarrow$  Pd(PPh<sub>3</sub>)<sub>4</sub>/NMM/HOAc/CHCl<sub>3</sub>

Step 3 3-maleimidopropionic acid



**Step 4** ↓ 85% TFA/5% TIS/5% thioanisole/5% phenol



### Example 23

#### Preparation of a Modified anti-SIV peptide

5           In this example, the peptide SEQ ID NO:66 is synthesized and modified to include a linker and maleimide group according to the synthesis scheme set forth below. As reported in U.S. Pat. Nos. 6,013,236 and 6,020,459, SEQ ID NO:66 exhibits potent antiviral activity as a crude peptide against simian immunodeficiency virus (SIV).

10           Solid phase peptide synthesis of the modified peptide on a 100  $\mu$ mole scale is performed using manual solid-phase synthesis, a Symphony Peptide Synthesizer and Fmoc protected Rink Amide MBHA. The following protected amino acids are sequentially added to resin: Fmoc-Asn(Trt)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Leu-OH, Fmoc-Glu(tBu)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Met-OH, Fmoc-Asn(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Ile-OH, Fmoc-Gln(Trt)-OH, Fmoc-Ala-OH, Fmoc-Glu(tBu)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Leu-OH, Fmoc-Leu-OH, Fmoc-Ala-OH, Fmoc-Thr(tBu)-OH, Fmoc-Ile-OH, Fmoc-Asn(Trt)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Leu-OH, Fmoc-Phe-OH, Fmoc-Asp(tBu)-OH, Fmoc-Val-OH, Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Glu(tBu)-OH Boc-Lys(Aloc)-OH,. They are dissolved in *N,N*-dimethylformamide (DMF) and, according to the sequence, activated using *O*-benzotriazol-1-yl-*N,N,N',N'*-tetramethyl-uronium hexafluorophosphate (HBTU) and Diisopropylethylamine (DIEA). Removal of the Fmoc protecting group is achieved using a solution of 20% (V/V) piperidine in *N,N*-dimethylformamide (DMF) for 20 minutes (step 1). The selective deprotection of the Lys (Aloc) group is performed manually and accomplished by treating the resin with a solution of 3 eq of  $\text{Pd}(\text{PPh}_3)_4$  dissolved in 5 mL of

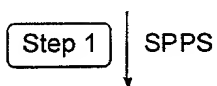
25

30  $\text{CHCl}_3$ :NMM:HOAc (18:1:0.5) for 2 h (Step 2). The resin is then washed with  $\text{CHCl}_3$  (6 x 5 mL), 20% HOAc in DCM (6 x 5 mL), DCM (6 x 5 mL), and DMF (6

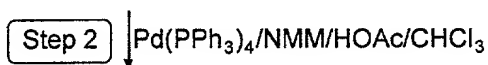
x 5 mL). The synthesis is then re-automated for the addition of the 3-  
maleimidopropionic acid (Step 3). Between every coupling, the resin is washed 3  
times with *N,N*-dimethylformamide (DMF) and 3 times with isopropanol. The  
peptide is cleaved from the resin using 85% TFA/5% TIS/5% thioanisole and 5%  
5 phenol, followed by precipitation by dry-ice cold Et<sub>2</sub>O (Step 4). The product is  
purified by preparative reversed phased HPLC using a Varian (Rainin) preparative  
binary HPLC system: gradient elution of 30-55% B (0.045% TFA in H<sub>2</sub>O (A) and  
0.045% TFA in CH<sub>3</sub>CN (B)) over 180 min at 9.5 mL/min using a Phenomenex  
Luna 10 μ phenyl-hexyl, 21 mm x 25 cm column and UV detector (Varian  
10 Dynamax UVD II) at λ 214 and 254 nm to afford the desired modified peptide  
(i.e., DAC) in >95% purity, as determined by RP-HPLC.

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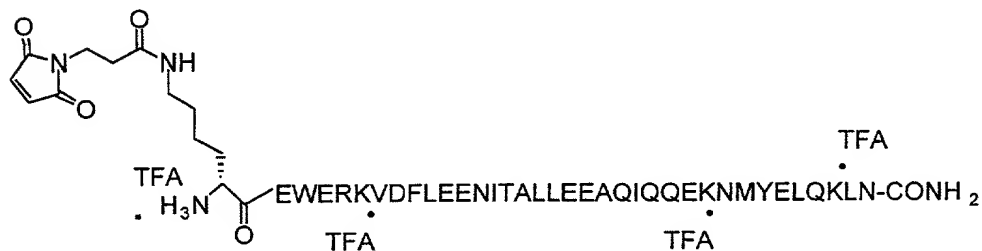
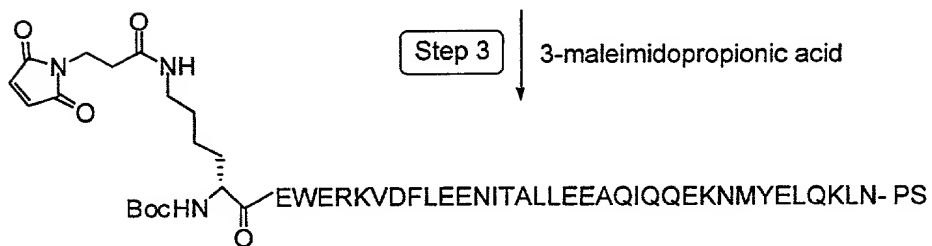
Fmoc-Rink Amide MBHA Resin



Boc-Lys(Aloc)-EWERKVDFLEENITALLEEAIQQEKNMYELQKLN-PS



Boc-Lys-EWERKVDFLEENITALLEEAIQQEKNMYELQKLN-PS



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#### Example 24

##### Preparation of a Modified anti-SIV peptide

In this example, the peptide SEQ ID NO:67 is synthesized and modified to include a linker and maleimide group according to the synthesis scheme set forth below. As reported in U.S. Pat. Nos. 6,013,236 and 6,020,459, SEQ ID NO:67 exhibits potent antiviral activity as a crude peptide against simian immunodeficiency virus (SIV).

Solid phase peptide synthesis of the modified peptide on a 100  $\mu$ mole scale is performed using manual solid-phase synthesis, a Symphony Peptide Synthesizer and Fmoc protected Rink Amide MBHA. The following protected amino acids are sequentially added to resin: Fmoc-Lys(Aloc)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Leu-OH, Fmoc-Glu(tBu)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Met-OH, Fmoc-Asn(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Ile-OH, Fmoc-Gln(Trt)-OH, Fmoc-Ala-OH, Fmoc-Glu(tBu)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Leu-OH, Fmoc-Leu-OH, Fmoc-Ala-OH, Fmoc-Thr(tBu)-OH, Fmoc-Ile-OH, Fmoc-Asn(Trt)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Leu-OH, Fmoc-Phe-OH, Fmoc-Asp(tBu)-OH, Fmoc-Val-OH, Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Trp(Boc)-OH. They are dissolved in *N,N*-dimethylformamide (DMF) and, according to the sequence, activated using *O*-benzotriazol-1-yl-*N,N,N',N'*-tetramethyl-uronium hexafluorophosphate (HBTU) and Diisopropylethylamine (DIEA). Removal of the Fmoc protecting group is achieved using a solution of 20% (V/V) piperidine in *N,N*-dimethylformamide (DMF) for 20 minutes (step 1). The selective deprotection of the Lys (Aloc) group is performed manually and accomplished by treating the resin with a solution of 3 eq of  $\text{Pd}(\text{PPh}_3)_4$  dissolved in 5 mL of  $\text{CHCl}_3$ :NMM:HOAc (18:1:0.5) for 2 h (Step 2). The resin is then washed with  $\text{CHCl}_3$  (6 x 5 mL), 20% HOAc in DCM (6 x 5 mL), DCM (6 x 5 mL), and

DMF (6 x 5 mL). The synthesis is then re-automated for the addition of the 3-maleimidopropionic acid (Step 3). Between every coupling, the resin is washed 3 times with *N,N*-dimethylformamide (DMF) and 3 times with isopropanol. The peptide is cleaved from the resin using 85% TFA/5% TIS/5% thioanisole and 5% phenol, followed by precipitation by dry-ice cold Et<sub>2</sub>O (Step 4). The product is purified by preparative reversed phased HPLC using a Varian (Rainin) preparative binary HPLC system: gradient elution of 30-55% B (0.045% TFA in H<sub>2</sub>O (A) and 0.045% TFA in CH<sub>3</sub>CN (B)) over 180 min at 9.5 mL/min using a Phenomenex Luna 10 μ phenyl-hexyl, 21 mm x 25 cm column and UV detector (Varian Dynamax UVD II) at λ 214 and 254 nm to afford the desired modified peptide (i.e., DAC) in >95% purity, as determined by RP-HPLC.



Fmoc-Rink Amide MBHA Resin

Step 1 ↓ SPPS

Boc-WERKVDLFLEENITALLEEAIQQEKNMYELQKLNS-Lys(Alloc)-PS

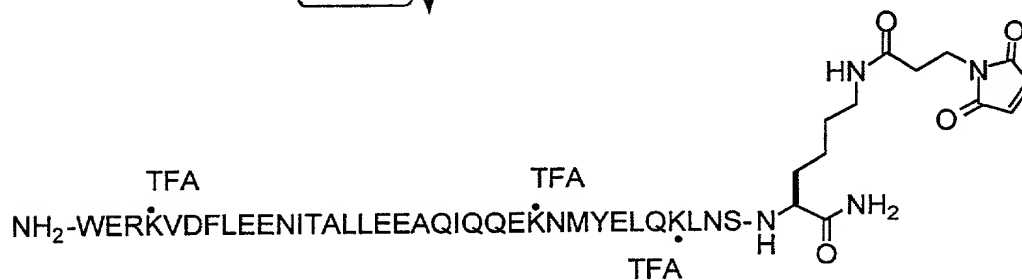
Step 2 ↓ Pd(PPh<sub>3</sub>)<sub>4</sub>/NMM/HOAc/CHCl<sub>3</sub>

Boc-WERKVDLFLEENITALLEEAIQQEKNMYELQKLNS-Lys-PS

Step 3 ↓ 3-maleimidopropionic acid



Step 4 ↓ 85% TFA/5% TIS/5% thioanisole/5% phenol



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### Example 25

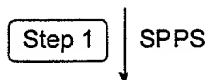
#### Preparation of a Modified anti-SIV peptide

In this example, the peptide SEQ ID NO:68 is synthesized and modified to include a linker and maleimide group according to the synthesis scheme set forth below. As reported in U.S. Pat. Nos. 6,013,236 and 6,020,459, SEQ ID NO:68 exhibits potent antiviral activity as a crude peptide against simian immunodeficiency virus (SIV).

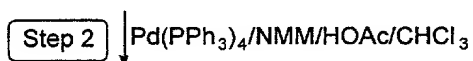
Solid phase peptide synthesis of the modified peptide on a 100  $\mu$ mole scale is performed using manual solid-phase synthesis, a Symphony Peptide Synthesizer and Fmoc protected Rink Amide MBHA. The following protected amino acids are sequentially added to resin: Fmoc-Trp(Boc)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Leu-OH, Fmoc-Glu(tBu)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Met-OH, Fmoc-Asn(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Ile-OH, Fmoc-Gln(Trt)-OH, Fmoc-Ala-OH, Fmoc-Glu(tBu)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Leu-OH, Fmoc-Leu-OH, Fmoc-Ala-OH, Fmoc-Thr(tBu)-OH, Fmoc-Ile-OH, Fmoc-Asn(Trt)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Leu-OH, Fmoc-Phe-OH, Fmoc-Asp(tBu)-OH, Fmoc-Val-OH, Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Glu(tBu)-OH Boc-Lys(Aloc)-OH,. They are dissolved in *N,N*-dimethylformamide (DMF) and, according to the sequence, activated using *O*-benzotriazol-1-yl-*N,N,N,N*-tetramethyl-uronium hexafluorophosphate (HBTU) and Diisopropylethylamine (DIEA). Removal of the Fmoc protecting group is achieved using a solution of 20% (V/V) piperidine in *N,N*-dimethylformamide (DMF) for 20 minutes (step 1). The selective deprotection of the Lys (Aloc) group is performed manually and accomplished by treating the resin with a solution of 3 eq of Pd(PPh<sub>3</sub>)<sub>4</sub> dissolved in 5 mL of CHCl<sub>3</sub>:NMM:HOAc (18:1:0.5) for 2 h (Step 2). The resin is then washed with CHCl<sub>3</sub> (6 x 5 mL), 20% HOAc in DCM (6 x 5 mL), DCM (6 x 5 mL), and

DMF (6 x 5 mL). The synthesis is then re-automated for the addition of the 3-maleimidopropionic acid (Step 3). Between every coupling, the resin is washed 3 times with *N,N*-dimethylformamide (DMF) and 3 times with isopropanol. The peptide is cleaved from the resin using 85% TFA/5% TIS/5% thioanisole and 5% phenol, followed by precipitation by dry-ice cold Et<sub>2</sub>O (Step 4). The product is purified by preparative reversed phased HPLC using a Varian (Rainin) preparative binary HPLC system: gradient elution of 30-55% B (0.045% TFA in H<sub>2</sub>O (A) and 0.045% TFA in CH<sub>3</sub>CN (B)) over 180 min at 9.5 mL/min using a Phenomenex Luna 10 μ phenyl-hexyl, 21 mm x 25 cm column and UV detector (Varian Dynamax UVD II) at λ 214 and 254 nm to afford the desired modified peptide (i.e., DAC) in >95% purity, as determined by RP-HPLC.

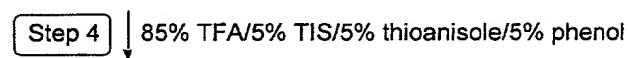
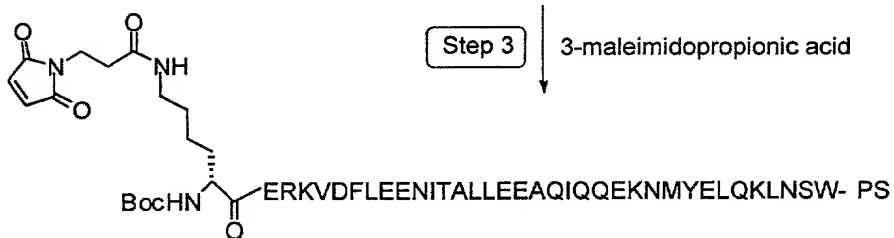
Fmoc-Rink Amide MBHA Resin



Boc-Lys(Aloc)-ERKVDLFLEENITALLEEAIQQEKNMYELQKLNSW -PS



Boc-Lys-ERKVDLFLEENITALLEEAIQQEKNMYELQKLNSW- PS



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## Example 26

### Preparation of a Modified anti-SIV peptide

In this example, the peptide SEQ ID NO:69 is synthesized and modified to include a linker and maleimide group according to the synthesis scheme set forth below. As reported in U.S. Pat. Nos. 6,013,236 and 6,020,459, SEQ ID NO:69 exhibits potent antiviral activity as a crude peptide against simian immunodeficiency virus (SIV).

Solid phase peptide synthesis of the modified peptide on a 100  $\mu$ mole scale is performed using manual solid-phase synthesis, a Symphony Peptide Synthesizer and Fmoc protected Rink Amide MBHA. The following protected amino acids are sequentially added to resin: Fmoc-Lys(Aloc)-OH, Fmoc-Asp(tBu)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Leu-OH, Fmoc-Glu(tBu)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Met-OH, Fmoc-Asn(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Ile-OH, Fmoc-Gln(Trt)-OH, Fmoc-Ala-OH, Fmoc-Glu(tBu)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Leu-OH, Fmoc-Leu-OH, Fmoc-Ala-OH, Fmoc-Thr(tBu)-OH, Fmoc-Ile-OH, Fmoc-Asn(Trt)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Leu-OH, Fmoc-Phe-OH, Fmoc-Asp(tBu)-OH, Fmoc-Val-OH, Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pbf)-OH. They are dissolved in *N,N*-dimethylformamide (DMF) and, according to the sequence, activated using *O*-benzotriazol-1-yl-*N,N,N',N'*-tetramethyl-uronium hexafluorophosphate (HBTU) and Diisopropylethylamine (DIEA). Removal of the Fmoc protecting group is achieved using a solution of 20% (V/V) piperidine in *N,N*-dimethylformamide (DMF) for 20 minutes (step 1). The selective deprotection of the Lys (Aloc) group is performed manually and accomplished by treating the resin with a solution of 3 eq of Pd(PPh<sub>3</sub>)<sub>4</sub> dissolved in 5 mL of CHCl<sub>3</sub>:NMM:HOAc (18:1:0.5) for 2 h (Step 2). The resin is then washed with CHCl<sub>3</sub> (6 x 5 mL), 20% HOAc in DCM (6 x 5 mL), DCM (6 x 5 mL), and

DMF (6 x 5 mL). The synthesis is then re-automated for the addition of the 3-maleimidopropionic acid (Step 3). Between every coupling, the resin is washed 3 times with *N,N*-dimethylformamide (DMF) and 3 times with isopropanol. The peptide is cleaved from the resin using 85% TFA/5% TIS/5% thioanisole and 5% phenol, followed by precipitation by dry-ice cold Et<sub>2</sub>O (Step 4). The product is purified by preparative reversed phased HPLC using a Varian (Rainin) preparative binary HPLC system: gradient elution of 30-55% B (0.045% TFA in H<sub>2</sub>O (A) and 0.045% TFA in CH<sub>3</sub>CN (B)) over 180 min at 9.5 mL/min using a Phenomenex Luna 10 μ phenyl-hexyl, 21 mm x 25 cm column and UV detector (Varian Dynamax UVD II) at λ 214 and 254 nm to afford the desired modified peptide (i.e., DAC) in >95% purity, as determined by RP-HPLC.

### Fmoc-Rink Amide MBHA Resin

### Step 1

**SPPS**

**Boc-RKVDFLEENITALLEEAQIQQEKNMYELQKLNSWD-Lys(Alloc)-PS**

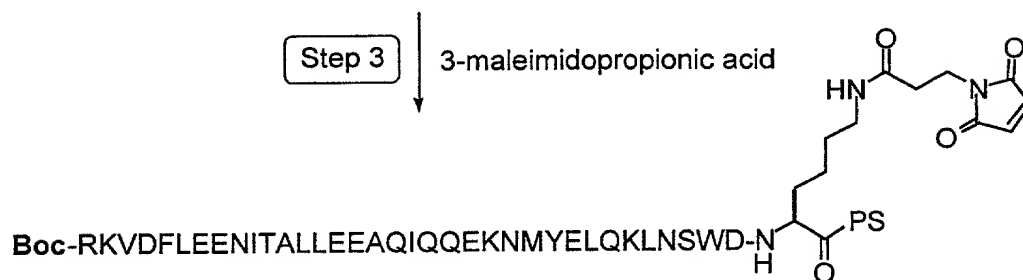
### Step 2

$\text{Pd}(\text{PPh}_3)_4/\text{NMM}/\text{HOAc}/\text{CHCl}_3$

**Boc-RKVDFLEENITALLEEAIQIQKEKNMYELQKLNSWD-Lys -PS**

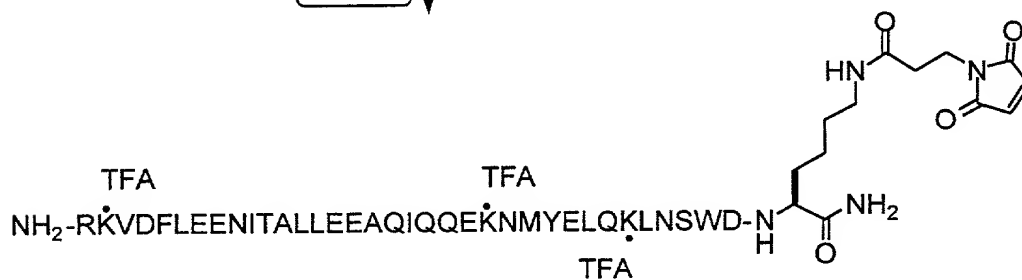
### Step 3

3-maleimidopropionic acid



### Step 4

85% TFA/5% TIS/5% thioanisole/5% phenol



### Example 27

#### Preparation of a Modified anti-SIV peptide

5 In this example, the peptide SEQ ID NO:70. is synthesized and modified to include a linker and maleimide group according to the synthesis scheme set forth below. As reported in U.S. Pat. Nos. 6,013,236 and 6,020,459, SEQ ID NO:70 exhibits potent antiviral activity as a crude peptide against simian immunodeficiency virus (SIV).

10

Solid phase peptide synthesis of the modified peptide on a 100  $\mu$ mole scale is performed using manual solid-phase synthesis, a Symphony Peptide Synthesizer and Fmoc protected Rink Amide MBHA. The following protected amino acids are sequentially added to resin: Fmoc-Val-OH, Fmoc-Asp(tBu)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Leu-OH, Fmoc-Glu(tBu)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Met-OH, Fmoc-Asn(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Ile-OH, Fmoc-Gln(Trt)-OH, Fmoc-Ala-OH, Fmoc-Glu(tBu)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Leu-OH, Fmoc-Leu-OH, Fmoc-Ala-OH, Fmoc-Thr(tBu)-OH, Fmoc-Ile-OH, Fmoc-Asn(Trt)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Leu-OH, Fmoc-Phe-OH, Fmoc-Asp(tBu)-OH, Fmoc-Val-OH, Fmoc-Lys(Boc)-OH, Boc-Lys(Aloc)-OH,. They are dissolved in *N,N*-dimethylformamide (DMF) and, according to the sequence, activated using O-benzotriazol-1-yl-*N, N, N', N'*-tetramethyl-uronium hexafluorophosphate (HBTU) and Diisopropylethylamine (DIEA). Removal of the Fmoc protecting group is achieved using a solution of 20% (V/V) piperidine in *N,N*-dimethylformamide (DMF) for 20 minutes (step 1). The selective deprotection of the Lys (Aloc) group is performed manually and accomplished by treating the resin with a solution of 3 eq of Pd(PPh<sub>3</sub>)<sub>4</sub> dissolved in 5 mL of

30

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CHCl<sub>3</sub>:NMM:HOAc (18:1:0.5) for 2 h (Step 2). The resin is then washed with CHCl<sub>3</sub> (6 x 5 mL), 20% HOAc in DCM (6 x 5 mL), DCM (6 x 5 mL), and DMF (6 x 5 mL). The synthesis is then re-automated for the addition of the 3-  
5 maleimidopropionic acid (Step 3). Between every coupling, the resin is washed 3 times with *N,N*-dimethylformamide (DMF) and 3 times with isopropanol. The peptide is cleaved from the resin using 85% TFA/5% TIS/5% thioanisole and 5% phenol, followed by precipitation by dry-ice cold Et<sub>2</sub>O (Step 4). The product is purified by preparative reversed phased HPLC using a Varian (Rainin) preparative binary HPLC system: gradient elution of 30-55% B (0.045% TFA in H<sub>2</sub>O (A) and 0.045% TFA in CH<sub>3</sub>CN (B)) over 180 min at 9.5 mL/min using a Phenomenex  
10 Luna 10 μ phenyl-hexyl, 21 mm x 25 cm column and UV detector (Varian Dynamax UVD II) at λ 214 and 254 nm to afford the desired modified peptide (i.e., DAC) in >95% purity, as determined by RP-HPLC.

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### Step 1

SPPS

### Step 2

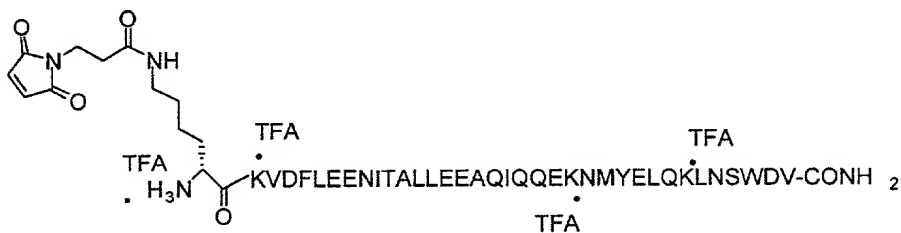
$\text{Pd(PPh}_3)_4/\text{NMM/HOAc/CHCl}_3$

### Step 3

CCNC(=O)CCN1C=CC(=O)N1C(C(=O)Nc1ccc(NC(=O)[K]VDFLEENITALLEEAIQQEKNMYELQKLNSWDV-PS)cc1)c1ccccc1

### Step 4

85% TFA/5% TIS/5% thioanisole/5% phenol



### Example 28

#### Preparation of a Modified anti-SIV peptide

5 In this example, the peptide SEQ ID NO:71 is synthesized and modified to include a linker and maleimide group according to the synthesis scheme set forth below. As reported in U.S. Pat. Nos. 6,013,236 and 6,020,459, SEQ ID NO:71 exhibits potent antiviral activity as a crude peptide against simian immunodeficiency virus (SIV).

10 Solid phase peptide synthesis of the modified peptide on a 100  $\mu$ mole scale is performed using manual solid-phase synthesis, a Symphony Peptide Synthesizer and Fmoc protected Rink Amide MBHA. The following protected amino acids are sequentially added to resin: Fmoc-Lys(Aloc)-OH, Fmoc-Phe-OH, Fmoc-Val-OH, 15 Fmoc-Asp(tBu)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Leu-OH, Fmoc-Glu(tBu)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Met-OH, Fmoc-Asn(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Ile-OH, Fmoc-Gln(Trt)-OH, Fmoc-Ala-OH, Fmoc-Glu(tBu)-OH, 20 Fmoc-Glu(tBu)-OH, Fmoc-Leu-OH, Fmoc-Leu-OH, Fmoc-Ala-OH, Fmoc-Thr(tBu)-OH, Fmoc-Ile-OH, Fmoc-Asn(Trt)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Leu-OH, Fmoc-Phe-OH, Fmoc-Asp(tBu)-OH, Fmoc-Val-OH. They are dissolved in *N,N*-dimethylformamide (DMF) and, according to the sequence, activated using *O*-benzotriazol-1-yl-*N,N,N',N'*-tetramethyl-uronium 25 hexafluorophosphate (HBTU) and Diisopropylethylamine (DIEA). Removal of the Fmoc protecting group is achieved using a solution of 20% (V/V) piperidine in *N,N*-dimethylformamide (DMF) for 20 minutes (step 1). The selective deprotection of the Lys (Aloc) group is performed manually and accomplished by treating the resin with a solution of 3 eq of Pd(PPh<sub>3</sub>)<sub>4</sub> dissolved in 5 mL of 30 CHCl<sub>3</sub>:NMM:HOAc (18:1:0.5) for 2 h (Step 2). The resin is then washed with

- CHCl<sub>3</sub> (6 x 5 mL), 20% HOAc in DCM (6 x 5 mL), DCM (6 x 5 mL), and DMF (6 x 5 mL). The synthesis is then re-automated for the addition of the 3-
- 5 maleimidopropionic acid (Step 3). Between every coupling, the resin is washed 3 times with *N,N*-dimethylformamide (DMF) and 3 times with isopropanol. The peptide is cleaved from the resin using 85% TFA/5% TIS/5% thioanisole and 5% phenol, followed by precipitation by dry-ice cold Et<sub>2</sub>O (Step 4). The product is purified by preparative reversed phased HPLC using a Varian (Rainin) preparative binary HPLC system: gradient elution of 30-55% B (0.045% TFA in H<sub>2</sub>O (A) and 0.045% TFA in CH<sub>3</sub>CN (B)) over 180 min at 9.5 mL/min using a Phenomenex
- 10 Luna 10 μ phenyl-hexyl, 21 mm x 25 cm column and UV detector (Varian Dynamax UVD II) at λ 214 and 254 nm to afford the desired modified peptide (i.e., DAC) in >95% purity, as determined by RP-HPLC.

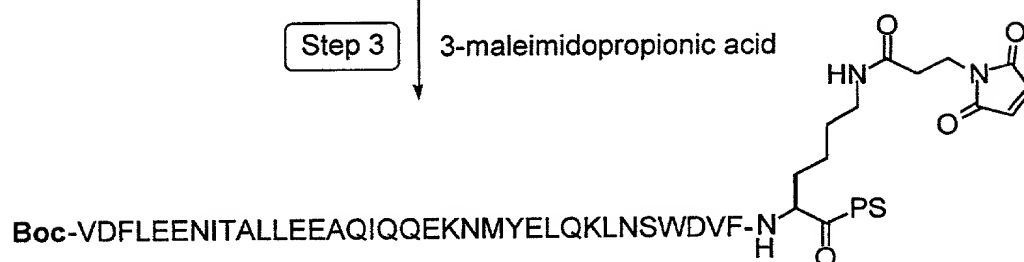
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Step 1 ↓ SPPS

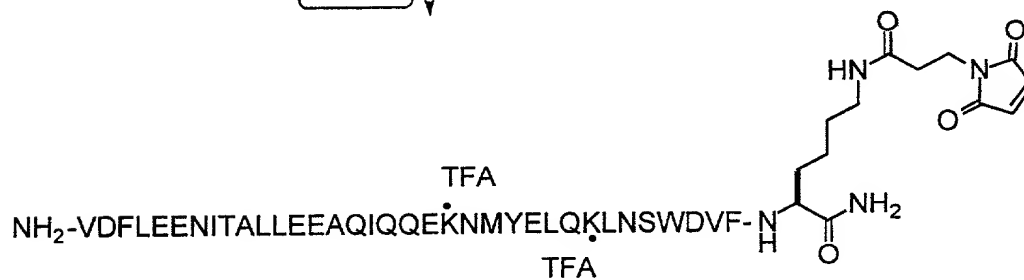
Step 2 ↓ Pd(PPh<sub>3</sub>)<sub>4</sub>/NMM/HOAc/CHCl<sub>3</sub>

Step 3

3-maleimidopropionic acid



Step 4 ↓ 85% TFA/5% TIS/5% thioanisole/5% phenol



### Example 29

#### Preparation of a Modified anti-SIV peptide

5 In this example, the peptide SEQ ID NO:72 is synthesized and modified to include a linker and maleimide group according to the synthesis scheme set forth below. As reported in U.S. Pat. Nos. 6,013,236 and 6,020,459, SEQ ID NO:72 exhibits potent antiviral activity as a crude peptide against simian immunodeficiency virus (SIV).

10 Solid phase peptide synthesis of the modified peptide on a 100  $\mu$ mole scale is performed using manual solid-phase synthesis, a Symphony Peptide Synthesizer and Fmoc protected Rink Amide MBHA. The following protected amino acids are sequentially added to resin: Fmoc-Lys(Aloc)-OH, Fmoc-Gly-OH, Fmoc-Phe-OH,  
15 Fmoc-Val-OH, Fmoc-Asp(tBu)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Leu-OH, Fmoc-Glu(tBu)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Met-OH, Fmoc-Asn(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Ile-OH, Fmoc-Gln(Trt)-OH, Fmoc-Ala-OH, Fmoc-  
20 Glu(tBu)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Leu-OH, Fmoc-Leu-OH, Fmoc-Ala-OH, Fmoc-Thr(tBu)-OH, Fmoc-Ile-OH, Fmoc-Asn(Trt)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Leu-OH, Fmoc-Phe-OH, Fmoc-Asp(tBu)-OH. They are dissolved in *N,N*-dimethylformamide (DMF) and, according to the sequence, activated using *O*-benzotriazol-1-yl-*N,N,N',N'*-tetramethyl-uronium  
25 hexafluorophosphate (HBTU) and Diisopropylethylamine (DIEA). Removal of the Fmoc protecting group is achieved using a solution of 20% (V/V) piperidine in *N,N*-dimethylformamide (DMF) for 20 minutes (step 1). The selective deprotection of the Lys (Aloc) group is performed manually and accomplished by treating the resin with a solution of 3 eq of Pd(PPh<sub>3</sub>)<sub>4</sub> dissolved in 5 mL of  
30 CHCl<sub>3</sub>:NMM:HOAc (18:1:0.5) for 2 h (Step 2). The resin is then washed with

CHCl<sub>3</sub> (6 x 5 mL), 20% HOAc in DCM (6 x 5 mL), DCM (6 x 5 mL), and DMF (6 x 5 mL). The synthesis is then re-automated for the addition of the 3-maleimidopropionic acid (Step 3). Between every coupling, the resin is washed 3 times with *N,N*-dimethylformamide (DMF) and 3 times with isopropanol. The peptide is cleaved from the resin using 85% TFA/5% TIS/5% thioanisole and 5% phenol, followed by precipitation by dry-ice cold Et<sub>2</sub>O (Step 4). The product is purified by preparative reversed phased HPLC using a Varian (Rainin) preparative binary HPLC system: gradient elution of 30-55% B (0.045% TFA in H<sub>2</sub>O (A) and 0.045% TFA in CH<sub>3</sub>CN (B)) over 180 min at 9.5 mL/min using a Phenomenex Luna 10 μ phenyl-hexyl, 21 mm x 25 cm column and UV detector (Varian Dynamax UVD II) at λ 214 and 254 nm to afford the desired modified peptide (i.e., DAC) in >95% purity, as determined by RP-HPLC.

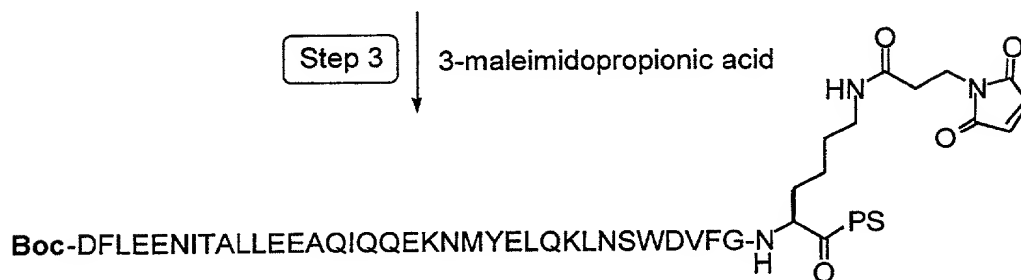
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Step 1 ↓ SPSS

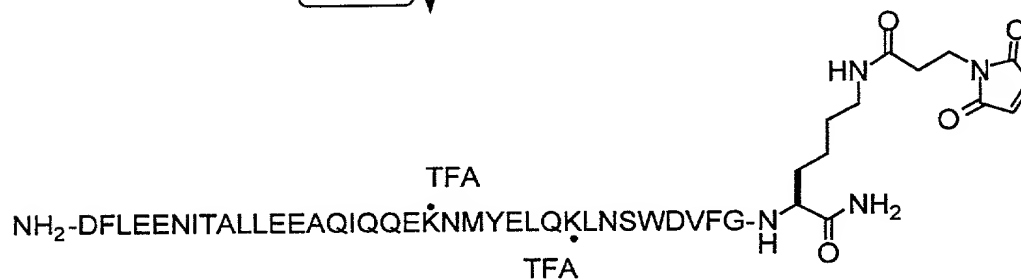
Step 2  $\downarrow$  Pd(PPh<sub>3</sub>)<sub>4</sub>/NMM/HOAc/CHCl<sub>3</sub>

Step 3

3-maleimidopropionic acid



Step 4 ↓ 85% TFA/5% TIS/5% thioanisole/5% phenol





### Example 30

#### Preparation of a Modified anti-SIV peptide

In this example, the peptide SEQ ID NO:73 is synthesized and modified to include a linker and maleimide group according to the synthesis scheme set forth below. As reported in U.S. Pat. Nos. 6,013,236 and 6,020,459, SEQ ID NO:73 exhibits potent antiviral activity as a crude peptide against simian immunodeficiency virus (SIV).

Solid phase peptide synthesis of the modified peptide on a 100  $\mu$ mole scale is performed using manual solid-phase synthesis, a Symphony Peptide Synthesizer and Fmoc protected Rink Amide MBHA. The following protected amino acids are sequentially added to resin: Fmoc-Lys(Aloc)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Gly-OH, Fmoc-Phe-OH, Fmoc-Val-OH, Fmoc-Asp(tBu)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Leu-OH, Fmoc-Glu(tBu)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Met-OH, Fmoc-Asn(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Ile-OH, Fmoc-Gln(Trt)-OH, Fmoc-Ala-OH, Fmoc-Glu(tBu)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Leu-OH, Fmoc-Leu-OH, Fmoc-Ala-OH, Fmoc-Thr(tBu)-OH, Fmoc-Ile-OH, Fmoc-Asn(Trt)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Leu-OH, Fmoc-Phe-OH. They are dissolved in *N,N*-dimethylformamide (DMF) and, according to the sequence, activated using *O*-benzotriazol-1-yl-*N,N,N',N'*-tetramethyl-uronium hexafluorophosphate (HBTU) and Diisopropylethylamine (DIEA). Removal of the Fmoc protecting group is achieved using a solution of 20% (V/V) piperidine in *N,N*-dimethylformamide (DMF) for 20 minutes (step 1). The selective deprotection of the Lys (Aloc) group is performed manually and accomplished by treating the resin with a solution of 3 eq of  $\text{Pd}(\text{PPh}_3)_4$  dissolved in 5 mL of  $\text{CHCl}_3$ :NMM:HOAc (18:1:0.5) for 2 h (Step 2). The resin is then washed with  $\text{CHCl}_3$  (6 x 5 mL), 20% HOAc in DCM (6 x 5 mL), DCM (6 x 5 mL), and DMF (6

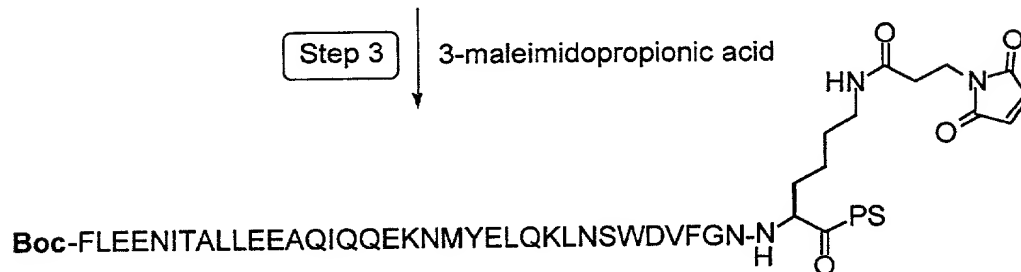
x 5 mL). The synthesis is then re-automated for the addition of the 3-maleimidopropionic acid (Step 3). Between every coupling, the resin is washed 3 times with *N,N*-dimethylformamide (DMF) and 3 times with isopropanol. The peptide is cleaved from the resin using 85% TFA/5% TIS/5% thioanisole and 5% phenol, followed by precipitation by dry-ice cold Et<sub>2</sub>O (Step 4). The product is purified by preparative reversed phased HPLC using a Varian (Rainin) preparative binary HPLC system: gradient elution of 30-55% B (0.045% TFA in H<sub>2</sub>O (A) and 0.045% TFA in CH<sub>3</sub>CN (B)) over 180 min at 9.5 mL/min using a Phenomenex Luna 10 μ phenyl-hexyl, 21 mm x 25 cm column and UV detector (Varian Dynamax UVD II) at λ 214 and 254 nm to afford the desired modified peptide (i.e., DAC) in >95% purity, as determined by RP-HPLC.

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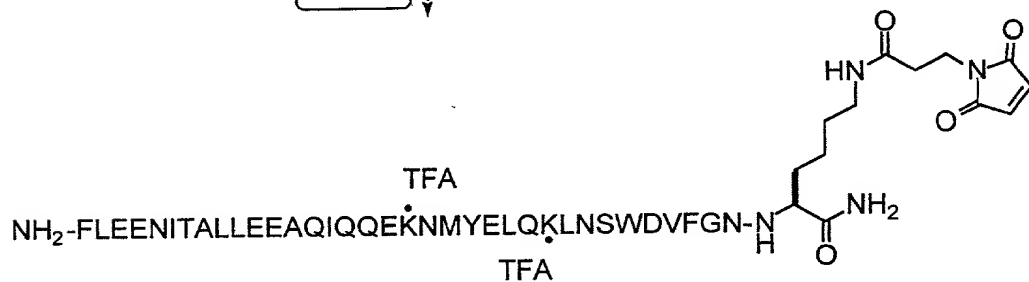
Step 1 SPPS

Step 2 | Pd(PPh<sub>3</sub>)<sub>4</sub>/NMM/HOAc/CHCl<sub>3</sub>

Step 3 3-maleimidopropionic acid



**Step 4** | 85% TFA/5% TIS/5% thioanisole/5% phenol



While certain embodiments of the invention have been described and exemplified, those having ordinary skill in the art will understand that the invention is not intended to be limited to the specifics of any of these embodiments, but is rather defined by the accompanying claims.

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**We claim:**

1. A modified anti-viral peptide comprising:  
a peptide that exhibits anti-viral activity, and  
5 a reactive group which is reactive with amino groups, hydroxyl groups, or  
thiol groups on blood components to form stable covalent bonds.
2. The modified peptide of claim 1 wherein said reactive group is a  
succinimidyl or a maleimido group.  
10
3. The modified peptide of claim 1 wherein said reactive group is a  
maleimido group which is reactive with a thiol group on a blood protein.
4. The modified peptide of claim 1 wherein said peptide is DP178 or  
15 DP107 or analogs thereof.
5. The modified peptide of claim 1 wherein said peptide exhibits anti-  
viral activity against human immunodeficiency virus (HIV).
- 20 6. The modified peptide of claim 5 wherein said peptide is selected  
from the group consisting of SEQ ID NO:1 to SEQ ID NO:9.
7. The modified peptide of claim 5 wherein said peptide is DP 178 or  
DP 107.  
25
8. The modified peptide of claim 1 wherein said peptide exhibits anti-  
viral activity against human respiratory syncytial virus (RSV).
9. The modified peptide of claim 8 wherein said peptide is selected  
30 from the group consisting of SEQ ID NO:10 to SEQ ID NO:30.

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18. The modified peptide of claim 17 wherein said peptide is selected from the group consisting of SEQ ID NO:63 to SEQ ID NO:73.

19. A composition for use in the prevention and/or treatment of acquired immune deficiency syndrome (AIDS) comprising a peptide that exhibits anti-viral activity against human immunodeficiency virus (HIV), modified with a reactive group which is reactive with amino groups, hydroxyl groups, or thiol groups on blood components to form stable covalent bonds.

20. The composition of claim 19 wherein said reactive group is a maleimido group which is reactive with a thiol group on a blood protein.

21. The composition of claim 20 wherein said peptide is DP178 or DP107 or analogs thereof.

22. A composition for use in the prevention and/or treatment of human respiratory syncytial virus (RSV) infection comprising a peptide that exhibits anti-viral activity against RSV, modified with a reactive group which is reactive with amino groups, hydroxyl groups, or thiol groups on blood components to form stable covalent bonds.

23. The composition of claim 22 wherein said reactive group is a maleimido group which is reactive with a thiol group on a blood protein.

24. The composition of claim 23 wherein said peptide is selected from the group consisting of SEQ ID NO:14 to SEQ ID NO:17 and SEQ ID NO:29.

25. A composition for use in the prevention and/or treatment of human parainfluenza virus (HPIV) infection comprising a peptide that exhibits anti-viral activity against human parainfluenza (HPIV), modified with a reactive group which is reactive with amino groups, hydroxyl groups, or thiol groups on blood components to form stable covalent bonds.

26. The composition of claim 25 wherein said reactive group is a maleimido group which is reactive with a thiol group on a blood protein.

5           27. The composition of claim 26 wherein said peptide is selected from the group consisting of SEQ ID NO: 35, SEQ ID NO:38 to SEQ ID NO:42, SEQ ID NO:52 and SEQ ID NO:58.

10           28. A composition for use in the prevention and/or treatment of measles virus (MeV) infection comprising a peptide that exhibits anti-viral activity against measles virus (MeV), modified with a reactive group which is reactive with amino groups, hydroxyl groups, or thiol groups on blood components to form stable covalent bonds.

15           29. The composition of claim 28 wherein said reactive group is a maleimido group which is reactive with a thiol group on a blood protein.

20           30. The composition of claim 29 wherein said peptide is selected from the group consisting of SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81 and SEQ ID NO:84.

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ABSTRACT OF THE DISCLOSURE

- 5      Peptides exhibiting anti-viral and anti-fusogenic activity are modified to provide greater stability and improved half-life *in vivo*. The selected peptides include fusion inhibitors DP178 and DP107 and related peptides and analogs thereof. The modified peptides are capable of forming covalent bonds with one or more blood components, preferably a mobile blood component.

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TABLE 2

DP178 CARBOXY TRUNCATIONS	
5	YTS YTSL YTSLI YTSLIH YTSLIHS
10	YTSLIHSL YTSLIHSLI YTSLIHSLIE YTSLIHSLIEE YTSLIHSLIEES
15	YTSLIHSLIEESQ YTSLIHSLIEESQN YTSLIHSLIEESQNNQ YTSLJHSLJEEESQNNQ YTSLIHSLIEESQNNQNE YTSLIHSLIEESQNNQNEK
20	YTSLIHSLIEESQNNQNEKN YTSLIHSLIEESQNNQNEKNE YTSLIHSLIEESQNNQNEKNEQ YTSLIHSLIEESQNNQNEKNEQE
25	YTSLIHSLIEESQNNQNEKNEQEL YTSLIHSLIEESQNNQNEKNEQELL YTSLIHSLIEESQNNQNEKNEQELLE YTSLIHSLIEESQNNQNEKNEQELLEL YTSLIHSLIEESQNNQNEKNEQELLELD
30	YTSLIHSLIEESQNNQNEKNEQELLELDK

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YTSLIHSLIEESQNQQEKNEQELLELDKW  
YTSLIHSLIEESQNQQEKNEQELLELDKWA  
YTSLIHSLIEESQNQQEKNEQELLELDKWAS  
YTSLIHSLIEESQNQQEKNEQELLELDKWASL  
5 YTSLIHSLIEESQNQQEKNEQELLELDKWASLW  
YTSLIHSLIEESQNQQEKNEQELLELDKWASLWN  
YTSLIHSLIEESQNQQEKNEQELLELDKWASLWNW  
YTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWF

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10 The one letter amino acid code of Table 1 is used.

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TABLE 3

DP178 AMINO TRUNCATIONS	
5	NWF
	WNWF
	LWNWF
	SLWNWF
10	ASLWNWF
	WASLWNWF
	KWASLWNWF
	DKWASLWNWF
	LDKWASLWNWF
15	ELDKWASLWNWF
	LELDKWASLWNWF
	LLELDKWASLWNWF
	ELLELDKWASLWNWF
	QELLELDKWASLWNWF
20	EQELLELDKWASLWNWF
	NEQELLELDKWASLWNWF
	KNEQELLELDKWASLWNWF
	EKNEQELLELDKWASLWNWF
	QEKNEQELLELDKWASLWNWF
25	QQEKNEQELLELDKWASLWNWF
	NQQEKNEQELLELDKWASLWNWF
	QNQQEKNEQELLELDKWASLWNWF
	SQNQQEKNEQELLELDKWASLWNWF
	ESQNQQEKNEQELLELDKWASLWNWF
30	EESQNQQEKNEQELLELDKWASLWNWF

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IEESQNQQEKNEQELLELDKWASLWNWF  
LIEESQNQQEKNEQELLELDKWASLWNWF  
SLIEESQNQQEKNEQELLELDKWASLWNWF  
HSLIEESQNQQEKNEQELLELDKWASLWNWF  
5 IHSLIEESQNQQEKNEQELLELDKWASLWNWF  
LIHSLIEESQNQQEKNEQELLELDKWASLWNWF  
SLIHSLIEESQNQQEKNEQELLELDKWASLWNWF  
TSLIHSLIEESQNQQEKNEQELLELDKWASLWNWF  
YTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWF

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The one letter amino acid code of Table 1 is used.

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TABLE 4

DP107 CARBOXY TRUNCATIONS	
5	NNL NNLL NNLLR NNLLRA NNLLRAI
10	NNLLRAIE NNLLRAIEA NNLLRAIEAQ NNLLRAIEAQQ NNLLRAIEAQQH
15	NNLLRAIEAQQHL NNLLRAIEAQQHLL NNLLRAIEAQQHLLQ NNLLRAIEAQQHLLQL NNLLRAIEAQQHLLQLT
20	NNLLRAIEAQQHLLQLTV NNLLRAIEAQQHLLQLTVW NNLLRAIEAQQHLLQLTVWQ NNLLRAIEAQQHLLQLTVWQI NNLLRAIEAQQHLLQLTVWQIK
25	NNLLRAIEAQQHLLQLTVWQIKQ NNLLRAIEAQQHLLQLTVWQIKQL NNLLRAIEAQQHLLQLTVWQIKQLQ NNLLRAIEAQQHLLQLTVWQIKQLQA NNLLRAIEAQQHLLQLTVWQIKQLQAR
30	NNLLRAIEAQQHLLQLTVWQIKQLQARI

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NNLLRAIEAQQHLLQLTVWQIKQLQARIL  
NNLLRAIEAQQHLLQLTVWQIKQLQARILA  
NNLLRAIEAQQHLLQLTVWQIKQLQARILAV  
NNLLRAIEAQQHLLQLTVWQIKQLQARILAVE  
5 NNLLRAIEAQQHLLQLTVWQIKQLQARILAV  
NNLLRAIEAQQHLLQLTVWQIKQLQARILAVERY  
NNLLRAIEAQQHLLQLTVWQIKQLQARILAVERYL  
NNLLRAIEAQQHLLQLTVWQIKQLQARILAVERYLK  
NNLLRAIEAQQHLLQLTVWQIKQLQARILAVERYLKD  
10 NNLLRAIEAQQHLLQLTVWQIKQLQARILAVERYLKDQ

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The one letter amino acid code of Table 1 is used.

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TABLE 5

DP107 AMINO TRUNCATIONS	
5	KDQ LKDQ YLKDQ RYLKDQ ERYLKDQ
10	VERYLKDQ AVERYLKDQ LAVERYLKDQ ILAVERYLKDQ RILAVERYLKDQ
15	ARILAVERYLKDQ QARILAVERYLKDQ LQARILAVERYLKDQ QLQARILAVERYLKDQ KQLQARILAVERYLKDQ
20	IKQLQARILAVERYLKDQ QIKQLQARILAVERYLKDQ WQIKQLQARILAVERYLKDQ VWQIKQLQARILAVERYLKDQ TVWQIKQLQARILAVERYLKDQ
25	LTVWQIKQLQARILAVERYLKDQ QLTVWQIKQLQARILAVERYLKDQ LQLTVWQIKQLQARILAVERYLKDQ LLQLTVWQIKQLQARILAVERYLKDQ HLLQLTVWQIKQLQARILAVERYLKDQ
30	QHLLQLTVWQIKQLQARILAVERYLKDQ



QQHLLQLTVWQIKQLQARILAVERYLKDQ  
AQHLLQLTVWQIKQLQARILAVERYLKDQ  
EAQQHLLQLTVWQIKQLQARILAVERYLKDQ  
IEAQQHLLQLTVWQIKQLQARILAVERYLKDQ  
5 AIEAQQHLLQLTVWQIKQLQARILAVERYLKDQ  
RAIEAQQHLLQLTVWQIKQLQARILAVERYLKDQ  
LRAIEAQQHLLQLTVWQIKQLQARILAVERYLKDQ  
LLRAIEAQQHLLQLTVWQIKQLQARILAVERYLKDQ  
NLLRAIEAQQHLLQLTVWQIKQLQARILAVERYLKDQ  
10 NNLLRAIEAQQHLLQLTVWQIKQLQARILAVERYLKDQ

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The one letter amino acid code of Table 1 is used.

005060" E E E E E E E E E E

TABLE 6

HIV-2 <sub>NH2</sub> DP178 analog carboxy truncations.	
5	LEA LEAN LEANI LEANIS LEANISQ
10	LEANISQS LEANISQSL LEANISQSLE LEANISQSLEQ LEANISQSLEQA
15	LEANISQSLEQAQ LEANISQSLEQAQI LEANISQSLEQAQIQ LEANISQSLEQAQIQQ LEANISQSLEQAQIQQE
20	LEANISQSLEQAQIQQEK LEANISQSLEQAQIQQEKN LEANISQSLEQAQIQQEKNM LEANISQSLEQAQIQQEKNMY LEANISQSLEQAQIQQEKNMYE
25	LEANISQSLEQAQIQQEKNMYEL LEANISQSLEQAQIQQEKNMYELQ LEANISQSLEQAQIQQEKNMYELQK LEANISQSLEQAQIQQEKNMYELQKL LEANISQSLEQAQIQQEKNMYELQKLN
30	LEANISQSLEQAQIQQEKNMYELQKLNS

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LEANISQSLEQAQIQQEKNMYELQKLNSW  
LEANISQSLEQAQIQQEKNMYELQKLNSWD  
LEANISQSLEQAQIQQEKNMYELQKLNSWDV  
LEANISQSLEQAQIQQEKNMYELQKLNSWDVF  
5 LEANISQSLEQAQIQQEKNMYELQKLNSWDVFT  
LEANISQSLEQAQIQQEKNMYELQKLNSWDVFTN  
LEANISQSLEQAQIQQEKNMYELQKLNSWDVFTNW  
LEANISQSLEQAQIQQEKNMYELQKLNSWDVFTNWL

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10 The one letter amino acid code of Table 1 is used.

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TABLE 7

HIV-2 <sub>NIH</sub> DP178 analog amino truncations.	
5	NWL TNWL FTNWL VFTNWL DVFTNWL
10	WDVFTNWL SWDVFTNWL NSWDVFTNWL LNSWDVFTNWL KLNSWDVFTNWL
15	QKLNSWDVFTNWL LQKLNSWDVFTNWL ELQKLNSWDVFTNWL YELQKLNSWDVFTNWL MYELQKLNSWDVFTNWL
20	NMYELQKLNSWDVFTNWL KNMYELQKLNSWDVFTNWL EKNMYELQKLNSWDVFTNWL QEKNMYELQKLNSWDVFTNWL QQEKNMYELQKLNSWDVFTNWL
25	IQQEKNMYELQKLNSWDVFTNWL QIQQEKNMYELQKLNSWDVFTNWL AQIQQEKNMYELQKLNSWDVFTNWL QAQIQQEKNMYELQKLNSWDVFTNWL EQAQIQQEKNMYELQKLNSWDVFTNWL
30	LEAQIQQEKNMYELQKLNSWDVFTNWL

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SLEQAQIQQEKNMYELQKLNSWDVFTNWL  
QSLEQAQIQQEKNMYELQKLNSWDVFTNWL  
SQSLEQAQIQQEKNMYELQKLNSWDVFTNWL  
ISQSLEQAQIQQEKNMYELQKLNSWDVFTNWL  
5 NISQSLEQAQIQQEKNMYELQKLNSWDVFTNWL  
ANISQSLEQAQIQQEKNMYELQKLNSWDVFTNWL  
EANISQSLEQAQIQQEKNMYELQKLNSWDVFTNWL  
LEANISQSLEQAQIQQEKNMYELQKLNSWDVFTNWL

---

10 The one letter amino acid code of Table 1 is used.

005060" EEE2360

TABLE 8

RESPIRATORY SYNCYTIAL VIRUS (RSV) DP107 F2 REGION ANALOG CARBOXY TRUNCATIONS	
5	YTS
	YTSV
	YTSVI
	YTSVIT
10	YTSVITI
	YTSVITIE
	YTSVITIEL
	YTSVITIELS
	YTSVITIELSN
15	YTSVITIELSNI
	YTSVITIELSNIK
	YTSVITIELSNIKE
	YTSVITIELSNIKEN
	YTSVITIELSNIKENK
20	YTSVITIELSNIKENKC
	YTSVITIELSNIKENKCN
	YTSVITIELSNIKENKCNG
	YTSVITIELSNIKENKCNGT
	YTSVITIELSNIKENKCNGTD
25	YTSVITIELSNIKENKCNGTDA
	YTSVITIELSNIKENKCNGTDAK
	YTSVITIELSNIKENKCNGTDAKV
	YTSVITIELSNIKENKCNGTDAKVK
	YTSVITIELSNIKENKCNGTDAKVKL
30	YTSVITIELSNIKENKCNGTDAKVKLI

YTSVITIELSNIKENKCNGTDAKVKLIK  
YTSVITIELSNIKENKCNGTDAKVKLIKQ  
YTSVITIELSNIKENKCNGTDAKVKLIKQE  
YTSVITIELSNIKENKCNGTDAKVKLIKQEL  
5 YTSVITIELSNIKENKCNGTDAKVKLIKQELD  
YTSVITIELSNIKENKCNGTDAKVKLIKQELDK  
YTSVITIELSNIKENKCNGTDAKVKLIKQELDKY  
YTSVITIELSNIKENKCNGTDAKVKLIKQELDKYK  
YTSVITIELSNIKENKCNGTDAKVKLIKQELDKYKN  
10 YTSVITIELSNIKENKCNGTDAKVKLIKQELDKYKNA  
YTSVITIELSNIKENKCNGTDAKVKLIKQELDKYKNAV  
YTSVITIELSNIKENKCNGTDAKVKLIKQELDKYKNAVT  
YTSVITIELSNIKENKCNGTDAKVKLIKQELDKYKNAVTE  
YTSVITIELSNIKENKCNGTDAKVKLIKQELDKYKNAVTEL  
15 YTSVITIELSNIKENKCNGTDAKVKLIKQELDKYKNAVTELQ  
YTSVITIELSNIKENKCNGTDAKVKLIKQELDKYKNAVTELQL  
YTSVITIELSNIKENKCNGTDAKVKLIKQELDKYKNAVTELQLL  
YTSVITIELSNIKENKCNGTDAKVKLIKQELDKYKNAVTELQLLM  
YTSVITIELSNIKENKCNGTDAKVKLIKQELDKYKNAVTELQLLMQ  
20 YTSVITIELSNIKENKCNGTDAKVKLIKQELDKYKNAVTELQLLMQS  
YTSVITIELSNIKENKCNGTDAKVKLIKQELDKYKNAVTELQLLMQST

---

The one letter amino acid code of Table 1 is used.

005060"EE5E2360

TABLE 9

RESPIRATORY SYNCYTIAL VIRUS (RSV) DP107 F2 REGION ANALOG AMINO TRUNCATIONS	
5	QST MQST LMQST LLMQST
10	QLLMQST LQLLMQST ELQLLMQST TELQLLMQST VTELQLLMQST
15	AVTELQLLMQST NAVTELQLLMQST KNAVTELQLLMQST YKNAVTELQLLMQST KYKNAVTELQLLMQST
20	DKYKNAVTELQLLMQST LDKYKNAVTELQLLMQST ELDKYKNAVTELQLLMQST QELDKYKNAVTELQLLMQST KQELDKYKNAVTELQLLMQST
25	IKQELDKYKNAVTELQLLMQST LIKQELDKYKNAVTELQLLMQST KLIKQELDKYKNAVTELQLLMQST VKLIKQELDKYKNAVTELQLLMQST KVKLIKQELDKYKNAVTELQLLMQST
30	AKVKLIKQELDKYKNAVTELQLLMQST

005060" EEE2960



005060"EE6E2960

DAKVKLIKQELDKYKNAVTELQLLMQST  
TDAKVKLIKQELDKYKNAVTELQLLMQST  
GTDAKVKLIKQELDKYKNAVTELQLLMQST  
NGTDAKVKLIKQELDKYKNAVTELQLLMQST  
5 CNGTDAKVKLIKQELDKYKNAVTELQLLMQST  
KCNGTDAKVKLIKQELDKYKNAVTELQLLMQST  
NKCNGTDAKVKLIKQELDKYKNAVTELQLLMQST  
KENKCNGTDAKVKLIKQELDKYKNAVTELQLLMQST  
IKENKCNGTDAKVKLIKQELDKYKNAVTELQLLMQST  
10 NIKENKCNGTDAKVKLIKQELDKYKNAVTELQLLMQST  
SNIKENKCNGTDAKVKLIKQELDKYKNAVTELQLLMQST  
LSNIKENKCNGTDAKVKLIKQELDKYKNAVTELQLLMQST  
ELSNIKENKCNGTDAKVKLIKQELDKYKNAVTELQLLMQST  
IELSNIKENKCNGTDAKVKLIKQELDKYKNAVTELQLLMQST  
15 TIELSNIKENKCNGTDAKVKLIKQELDKYKNAVTELQLLMQST  
ITIELSNIKENKCNGTDAKVKLIKQELDKYKNAVTELQLLMQST  
VITIELSNIKENKCNGTDAKVKLIKQELDKYKNAVTELQLLMQST  
SVITIELSNIKENKCNGTDAKVKLIKQELDKYKNAVTELQLLMQST  
TSVITIELSNIKENKCNGTDAKVKLIKQELDKYKNAVTELQLLMQST  
20

---

The one letter amino acid code of Table 1 is used.

TABLE 10

---

RESPIRATORY SYNCYTIAL VIRUS (RSV) F1 DP178  
REGION ANALOG CARBOXY TRUNCATIONS

---

5	FYD
	FYDP
	FYDPL
	FYDPLV
10	FYDPLVF
	FYDPLVFP
	FYDPLVFPS
	FYDPLVFPSD
	FYDPLVFPSDE
15	FYDPLVFPSDEF
	FYDPLVFPSDEFD
	FYDPLVFPSDEFDA
	FYDPLVFPSDEFDAS
	FYDPLVFPSDEFDASI
20	FYDPLVFPSDEFDASIS
	FYDPLVFPSDEFDASISQ
	FYDPLVFPSDEFDASISQV
	FYDPLVFPSDEFDASISQVN
	FYDPLVFPSDEFDASISQVNE
25	FYDPLVFPSDEFDASISQVNEK
	FYDPLVFPSDEFDASISQVNEKI
	FYDPLVFPSDEFDASISQVNEKIN
	FYDPLVFPSDEFDASISQVNEKINQ
	FYDPLVFPSDEFDASISQVNEKINQS
30	FYDPLVFPSDEFDASISQVNEKINQSL

FYDPLVFPSDEFDASISQVNEKINQSLA  
FYDPLVFPSDEFDASISQVNEKINQSLAF  
FYDPLVFPSDEFDASISQVNEKINQSLAFI  
FYDPLVFPSDEFDASISQVNEKINQSLAFIR  
5 FYDPLVFPSDEFDASISQVNEKINQSLAFIRK  
FYDPLVFPSDEFDASISQVNEKINQSLAFIRKS  
FYDPLVFPSDEFDASISQVNEKINQSLAFIRKSD  
FYDPLVFPSDEFDASISQVNEKINQSLAFIRKSDE  
FYDPLVFPSDEFDASISQVNEKINQSLAFIRKSDEL  
10 FYDPLVFPSDEFDASISQVNEKINQSLAFIRKSDELL

---

The one letter amino acid code of Table 1 is used.

005050"EE32350

TABLE 11

RESPIRATORY SYNCYTIAL VIRUS (RSV) F1 DP178	
REGION ANALOG AMINO TRUNCATIONS	
5	DELL
	SDELL
	KSDELL
	RKSDELL
10	IRKSDELL
	FIRKSDELL
	AFIRKSDELL
	LAFIRKSDELL
	SLAFIRKSDELL
15	QSLAFIRKSDELL
	NQSLAFIRKSDELL
	INQSLAFIRKSDELL
	KINQSLAFIRKSDELL
	EKINQSLAFIRKSDELL
20	NEKINQSLAFIRKSDELL
	VNEKINQSLAFIRKSDELL
	QVNEKINQSLAFIRKSDELL
	SQVNEKINQSLAFIRKSDELL
	ISQVNEKINQSLAFIRKSDELL
25	SISQVNEKINQSLAFIRKSDELL
	ASISQVNEKINQSLAFIRKSDELL
	DASISQVNEKINQSLAFIRKSDELL
	FDASISQVNEKINQSLAFIRKSDELL
	EFDASISQVNEKINQSLAFIRKSDELL
30	DEFDASISQVNEKINQSLAFIRKSDELL

005060" ESE 2360

SDEFDASISQVNEKINQSLAFIRKSDELL  
PSDEFDASISQVNEKINQSLAFIRKSDELL  
FPSDEFDASISQVNEKINQSLAFIRKSDELL  
VFPSDEFDASISQVNEKINQSLAFIRKSDELL  
5 LVFPSDEFDASISQVNEKINQSLAFIRKSDELL  
PLVFPSDEFDASISQVNEKINQSLAFIRKSDELL  
DPLVFPSDEFDASISQVNEKINQSLAFIRKSDELL  
YDPLVFPSDEFDASISQVNEKINQSLAFIRKSDELL

---

10 The one letter amino acid code of Table 1 is used.

005060"EE2260

HUMAN PARAINFLUENZA VIRUS 3 (HPV3) F1 REGION DP178  
ANALOG CARBOXY TRUNCATIONS

5	ITL
	ITLN
	ITLNN
	ITLNNS
10	ITLNNSV
	ITLNNSVA
	ITLNNSVAL
	ITLNNSVALD
	ITLNNSVALDP
15	ITLNNSVALDPID
	ITLNNSVALDPID
	ITLNNSVALDPIDI
	ITLNNSVALDPIDIS
	ITLNNSVALDPIDISI
20	ITLNNSVALDPIDISIE
	ITLNNSVALDPIDISIEL
	ITLNNSVALDPIDISIELN
	ITLNNSVALDPIDISIELNK
	ITLNNSVALDPIDISIELNKA
25	ITLNNSVALDPIDISIELNKAK
	ITLNNSVALDPIDISIELNKAKS
	ITLNNSVALDPIDISIELNKAKSD
	ITLNNSVALDPIDISIELNKAKSDL
	ITLNNSVALDPIDISIELNKAKSDLE
30	ITLNNSVALDPIDISIELNKAKSDLEE

ITLNNSVALDPIDISIELNKA KSDLEES  
ITLNNSVALDPIDISIELNKA KSDLEESK  
ITLNNSVALDPIDISIELNKA KSDLEESKE  
ITLNNSVALDPIDISIELNKA KSDLEESKEW  
5 ITLNNSVALDPIDISIELNKA KSDLEESKEWI  
ITLNNSVALDPIDISIELNKA KSDLEESKEWIR  
ITLNNSVALDPIDISIELNKA KSDLEESKEWIRR  
ITLNNSVALDPIDISIELNKA KSDLEESKEWIRRS

---

10 The one letter amino acid code of Table 1 is used.

005060"EESEES

TABLE 13

HUMAN PARAINFLUENZA VIRUS 3 (HPV3) F1 REGION DP178	
ANALOG AMINO TRUNCATIONS	
5	RRS
	IRRS
	WIRRS
	EWIRRS
10	KEWIRRS
	SKEWIRRS
	ESKEWIRRS
	EESKEWIRRS
	LEESKEWIRRS
15	DLEESKEWIRRS
	SDLEESKEWIRRS
	KSDLEESKEWIRRS
	AKSDLEESKEWIRRS
	KAKSDLEESKEWIRRS
20	NKAKSDLEESKEWIRRS
	LNKAKSDLEESKEWIRRS
	ELNKAKSDLEESKEWIRRS
	IELNKAKSDLEESKEWIRRS
	SIELNKAKSDLEESKEWIRRS
25	ISIELNKAKSDLEESKEWIRRS
	DISIELNKAKSDLEESKEWIRRS
	IDISIELNKAKSDLEESKEWIRRS
	PIDISIELNKAKSDLEESKEWIRRS
	DPIDISIELNKAKSDLEESKEWIRRS
30	LDPIDISIELNKAKSDLEESKEWIRRS



ALDPIDISIELNKA KSDLEESKEWIRRS  
VALDPIDISIELNKA KSDLEESKEWIRRS  
SVALDPIDISIELNKA KSDLEESKEWIRRS  
NSVALDPIDISIELNKA KSDLEESKEWIRRS  
5 NNSVALDPIDISIELNKA KSDLEESKEWIRRS  
LNNSVALDPIDISIELNKA KSDLEESKEWIRRS  
TLNNSVALDPIDISIELNKA KSDLEESKEWIRRS

---

The one letter amino acid code of Table 1 is used.

005060 "E" 5E 2360

TABLE 14

HUMAN PARAINFLUENZA VIRUS 3 (HPV3) F1 REGION	
DP107 ANALOG CARBOXY TRUNCATIONS	
5	ALG
	ALGV
	ALGVA
	ALGVAT
10	ALGVATS
	ALGVATSA
	ALGVATSAQ
	ALGVATSAQI
	ALGVATSAQIT
15	ALGVATSAQITA
	ALGVATSAQITAA
	ALGVATSAQITAAV
	ALGVATSAQITAAVA
	ALGVATSAQITAVAL
20	ALGVATSAQITAVALV
	ALGVATSAQITAVALVE
	ALGVATSAQITAVALVEA
	ALGVATSAQITAVALVEAK
	ALGVATSAQITAVALVEAKQ
25	ALGVATSAQITAVALVEAKQA
	ALGVATSAQITAVALVEAKQAR
	ALGVATSAQITAVALVEAKQARS
	ALGVATSAQITAVALVEAKQARSD
	ALGVATSAQITAVALVEAKQARSDI
30	ALGVATSAQITAVALVEAKQARSDIE

005060 "EE2350

ALGVATSAQITA A VALVEAKQARSDIEK  
ALGVATSAQITA A VALVEAKQARSDIEKL  
ALGVATSAQITA A VALVEAKQARSDIEKLK  
ALGVATSAQITA A VALVEAKQARSDIEKLKE  
5 ALGVATSAQITA A VALVEAKQARSDIEKLKEA  
ALGVATSAQITA A VALVEAKQARSDIEKLKEAI  
ALGVATSAQITA A VALVEAKQARSDIEKLKEAIR

---

The one letter amino acid code of Table 1 is used.

005060"EESE2360

TABLE 15

HUMAN PARAINFLUENZA VIRUS 3 (HPV3) F1 REGION	
DP107 ANALOG AMINO TRUNCATIONS	
5	IRD
	AIRD
	EAIRD
	KEAIRD
10	LKEAIRD
	KLKEAIRD
	EKLKEAIRD
	IEKLKEAIRD
	DIEKLKEAIRD
15	SDIEKLKEAIRD
	RSDIEKLKEAIRD
	ARSDIEKLKEAIRD
	QARSDIEKLKEAIRD
	KQARSDIEKLKEAIRD
20	AKQARSDIEKLKEAIRD
	EAKQARSDIEKLKEAIRD
	VEAKQARSDIEKLKEAIRD
	LVEAKQARSDIEKLKEAIRD
	ALVEAKQARSDIEKLKEAIRD
25	VALVEAKQARSDIEKLKEAIRD
	AVALVEAKQARSDIEKLKEAIRD
	AAVALVEAKQARSDIEKLKEAIRD
	TAAVALVEAKQARSDIEKLKEAIRD
	ITAAVALVEAKQARSDIEKLKEAIRD
30	QITAAVALVEAKQARSDIEKLKEAIRD

005060" E5E2950

AQITA AVALVEAKQARSDIEKLKEAIRD  
SAQITA AVALVEAKQARSDIEKLKEAIRD  
TSAQITA AVALVEAKQARSDIEKLKEAIRD  
ATSAQITA AVALVEAKQARSDIEKLKEAIRD  
5 VATSAQITA AVALVEAKQARSDIEKLKEAIRD  
GVATSAQITA AVALVEAKQARSDIEKLKEAIRD  
LGVATSAQITA AVALVEAKQARSDIEKLKEAIRD

---

The one letter amino acid code of Table 1 is used.

005050" EEE2550

TABLE 16

ANTI-RESPIRATORY SYNCYTIAL VIRUS (RSV) PEPTIDES

5	TSVITIELSNIKENKCNGTDAKVKLIKQELDKYKN SVITIELSNIKENKCNGTDAKVKLIKQELDKYKNA VITIELSNIKENKCNGTDAKVKLIKQELDKYKNAV VAVSKVLHLEGEVNKIALSTNKAVVSLSNGVS AVSKVLHLEGEVNKIALSTNKAVVSLSNGVSV
10	VSKVLHLEGEVNKIALSTNKAVVSLSNGVSVL SKVLHLEGEVNKIALSTNKAVVSLSNGVSVLT KVLHLEGEVNKIALSTNKAVVSLSNGVSVLTS LEGEVNKIALSTNKAVVSLSNGVSVLTSKVLD GEVNKIALSTNKAVVSLSNGVSVLTSKVLDLK
15	EVNKIALSTNKAVVSLSNGVSVLTSKVLDLKN VNKIALSTNKAVVSLSNGVSVLTSKVLDLKNY NKIALSTNKAVVSLSNGVSVLTSKVLDLKNYI KIALSTNKAVVSLSNGVSVLTSKVLDLKNYID IALSTNKAVVSLSNGVSVLTSKVLDLKNYIDK
20	ALLSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQ VAVSKVLHLEGEVNKIALSTNKAVVSLSNGVS AVSKVLHLEGEVNKIALSTNKAVVSLSNGVSV VSKVLHLEGEVNKIALSTNKAVVSLSNGVSVL SKVLHLEGEVNKIALSTNKAVVSLSNGVSVLT
25	KVLHLEGEVNKIALSTNKAVVSLSNGVSVLTS LEGEVNKIALSTNKAVVSLSNGVSVLTSKVLD GEVNKIALSTNKAVVSLSNGVSVLTSKVLDLK EVNKIALSTNKAVVSLSNGVSVLTSKVLDLKN VNKIALSTNKAVVSLSNGVSVLTSKVLDLKNY
30	NKIALSTNKAVVSLSNGVSVLTSKVLDLKNYI

KIALLSTNKAVVSLSNGVSVLTSKVLDLKNYID  
IALLSTNKAVVSLSNGVSVLTSKVLDLKNYIDK  
ALLSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQ

---

- 5 The one letter amino acid code of Table 1 is used.

005060"EE3E2960

TABLE 17

ANTI-HUMAN PARAINFLUENZA VIRUS 3 (HPV3) PEPTIDES

5	TLNNSVALDPIDISIELNKA	SDLEESKEWIRRSN
	LNNSVALDPIDISIELNKA	SDLEESKEWIRRSNQ
	NNSVALDPIDISIELNKA	SDLEESKEWIRRSNQK
	NSVALDPIDISIELNKA	SDLEESKEWIRRSNQKL
	SVALDPIDISIELNKA	SDLEESKEWIRRSNQKLD
10	VALDPIDISIELNKA	SDLEESKEWIRRSNQKLDS
	ALDPIDISIELNKA	SDLEESKEWIRRSNQKLDSI
	LDPIDISIELNKA	SDLEESKEWIRRSNQKLDSIG
	DPIDISIELNKA	SDLEESKEWIRRSNQKLDSIGN
	PIDISIELNKA	SDLEESKEWIRRSNQKLDSIGNW
15	IDISIELNKA	SDLEESKEWIRRSNQKLDSIGNWH
	DISIELNKA	SDLEESKEWIRRSNQKLDSIGNWHQ
	ISIELNKA	SDLEESKEWIRRSNQKLDSIGNWHQS
	SIELNKA	SDLEESKEWIRRSNQKLDSIGNWHQSS
	IELNKA	SDLEESKEWIRRSNQKLDSIGNWHQSST
20	ELNKA	SDLEESKEWIRRSNQKLDSIGNWHQSSTT
	TAAVALVEAKQARSDIEKLKEAIRD	TNKAVQSVQS
	AVALVEAKQARSDIEKLKEAIRD	TNKAVQSVQSSI
	LVEAKQARSDIEKLKEAIRD	TNKAVQSVQSSIGNL
	VEAKQARSDIEKLKEAIRD	TNKAVQSVQSSIGNLI
25	EAKQARSDIEKLKEAIRD	TNKAVQSVQSSIGNLIV
	AKQARSDIEKLKEAIRD	TNKAVQSVQSSIGNLIVA
	KQARSDIEKLKEAIRD	TNKAVQSVQSSIGNLIVAI
	QARSDIEKLKEAIRD	TNKAVQSVQSSIGNLIVAIK
	ARSDIEKLKEAIRD	TNKAVQSVQSSIGNLIVAIKS
30	RSDIEKLKEAIRD	TNKAVQSVQSSIGNLIVAIKSV

005060" E552960



SDIEKLKEAIRDTNKAVQSVQSSIGNLIVAIKSVQ  
KLKEAIRDTNKAVQSVQSSIGNLIVAIKSVQDYVN  
LKEAIRDTNKAVQSVQSSIGNLIVAIKSVQDYVNK  
AIRDTNKAVQSVQSSIGNLIVAIKSVQDYVNKEIV

5

---

The one letter amino acid code of Table 1 is used.

005060"EE22960

TABLE 18

---

ANTI-SIMIAN IMMUNODEFICIENCY VIRUS (SIV) PEPTIDES

---

5	WQEWERKVD FLEENITALLEEAQIQQEKNMYELQK
	QEWERKVD FLEENITALLEEAQIQQEKNMYELQKL
	EWERKVD FLEENITALLEEAQIQQEKNMYELQKLN
	WERKVD FLEENITALLEEAQIQQEKNMYELQKLNS
	ERKVD FLEENITALLEEAQIQQEKNMYELQKLNSW
10	RKVD FLEENITALLEEAQIQQEKNMYELQKLNSWD
	KVD FLEENITALLEEAQIQQEKNMYELQKLNSWDV
	VDFLEENITALLEEAQIQQEKNMYELQKLNSWDVF
	DFLEENITALLEEAQIQQEKNMYELQKLNSWDVFG
	FLEENITALLEEAQIQQEKNMYELQKLNSWDVFGN

---

15

The one letter amino acid code of Table 1 is used.

005060" EEE2360

TABLE 19

---

ANTI-MEASLES VIRUS (MEV) PEPTIDES

---

5	LHRIDLGPISLERLDVGTNLGNIAKLEAKELL
	HRIDLGPISLERLDVGTNLGNIAKLEAKELLE
	RIDLGPISLERLDVGTNLGNIAKLEAKELLES
	IDLGPISLERLDVGTNLGNIAKLEAKELLESS
	DLGPISLERLDVGTNLGNIAKLEAKELLESSD
10	LGPPISLERLDVGTNLGNIAKLEAKELLESSDQ
	GPPISLERLDVGTNLGNIAKLEAKELLESSDQI
	PPISLERLDVGTNLGNIAKLEAKELLESSDQIL
	PISLERLDVGTNLGNIAKLEAKELLESSDQILR
	SLERLDVGTNLGNIAKLEAKELLESSDQILRSM
15	LERLDVGTNLGNIAKLEAKELLESSDQILRSMK

---

The one letter amino acid code of Table 1 is used.

005050" EEE2950

SEQUENCE LISTING

<110> Conjuchem, Inc.

<120> LONG LASTING FUSION PEPTIDE INHIBITORS OF VIRAL  
INFECTION

<130> REDC-1510

<140>

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<150> US 60/134,406

<151> 1999-05-17

<150> US 60/153,406

<151> 1999-09-10

<160> 86

<170> PatentIn Ver. 2.1

<210> 1

<211> 36

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
peptide

<400> 1

Tyr Thr Ser Leu Ile His Ser Leu Ile Glu Glu Ser Gln Asn Gln Gln  
1 5 10 15

Glu Lys Asn Glu Gln Glu Leu Leu Glu Leu Asp Lys Trp Ala Ser Leu  
20 25 30

Trp Asn Trp Phe  
35

<210> 2

<211> 38

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
peptide

<400> 2

Asn Asn Leu Leu Arg Ala Ile Glu Ala Gln Gln His Leu Leu Gln Leu  
1 5 10 15

Thr Val Trp Gln Ile Lys Gln Leu Gln Ala Arg Ile Leu Ala Val Glu  
20 25 30

Arg Tyr Leu Lys Asp Gln  
35

<210> 3

<211> 36

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
peptide

<400> 3

Tyr Thr Asn Thr Ile Tyr Thr Leu Leu Glu Glu Ser Gln Asn Gln Gln  
1 5 10 15

Glu Lys Asn Glu Gln Glu Leu Leu Glu Leu Asp Lys Trp Ala Ser Leu  
20 25 30

Trp Asn Trp Phe  
35

<210> 4

<211> 36

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
peptide

<400> 4

Tyr Thr Gly Ile Ile Tyr Asn Leu Leu Glu Glu Ser Gln Asn Gln Gln  
1 5 10 15

Glu Lys Asn Glu Gln Glu Leu Leu Glu Leu Asp Lys Trp Ala Asn Leu

20

25

30

Trp Asn Trp Phe

35

&lt;210&gt; 5

&lt;211&gt; 36

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: synthetic  
peptide

&lt;400&gt; 5

Tyr Thr Ser Leu Ile Tyr Ser Leu Leu Glu Lys Ser Gln Thr Gln Gln

1

5

10

15

Glu Lys Asn Glu Gln Glu Leu Leu Glu Leu Asp Lys Trp Ala Ser Leu

20

25

30

Trp Asn Trp Phe

35

&lt;210&gt; 6

&lt;211&gt; 36

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: synthetic  
peptide

&lt;400&gt; 6

Leu Glu Ala Asn Ile Ser Lys Ser Leu Glu Gln Ala Gln Ile Gln Gln

1

5

10

15

Glu Lys Asn Met Tyr Glu Leu Gln Lys Leu Asn Ser Trp Asp Ile Phe

20

25

30

Gly Asn Trp Phe

35

&lt;210&gt; 7

&lt;211&gt; 36

<212> PRT  
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
peptide

<400> 7

Leu Glu Ala Asn Ile Ser Gln Ser Leu Glu Gln Ala Gln Ile Gln Gln  
1 5 10 15

Glu Lys Asn Met Tyr Glu Leu Gln Lys Leu Asn Ser Trp Asp Val Phe  
20 25 30

Thr Asn Trp Leu  
35

<210> 8

<211> 41

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
peptide

<400> 8

Cys Gly Gly Asn Asn Leu Leu Arg Ala Ile Glu Ala Gln Gln His Leu  
1 5 10 15

Leu Gln Leu Thr Val Trp Gly Ile Lys Gln Leu Gln Ala Arg Ile Leu  
20 25 30

Ala Val Glu Arg Tyr Leu Lys Asp Gln  
35 40

<210> 9

<211> 38

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
peptide

<400> 9

Gln Gln Leu Leu Asp Val Val Lys Arg Gln Gln Glu Met Leu Arg Leu  
 1 5 10 15

Thr Val Trp Gly Thr Lys Asn Leu Gln Ala Arg Val Thr Ala Ile Glu  
 20 25 30

Lys Tyr Leu Lys Asp Gln  
 35

<210> 10  
 <211> 46  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: synthetic  
 peptide

<400> 10  
 Tyr Thr Ser Val Ile Thr Ile Glu Leu Ser Asn Ile Lys Glu Asn Lys  
 1 5 10 15

Cys Asn Gly Ala Lys Val Lys Leu Ile Lys Gln Glu Leu Asp Lys Tyr  
 20 25 30

Lys Asn Ala Val Thr Glu Leu Gln Leu Leu Met Gln Ser Thr  
 35 40 45

<210> 11  
 <211> 54  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: synthetic  
 peptide

<400> 11  
 Ala Ser Gly Val Ala Val Ser Lys Val Leu His Leu Glu Gly Glu Val  
 1 5 10 15

Asn Lys Ile Ala Leu Leu Ser Thr Asn Lys Ala Val Val Ser Leu Ser  
 20 25 30

Asn Gly Val Ser Val Leu Thr Ser Lys Val Leu Asp Leu Lys Asn Tyr  
 35 40 45

005060" E25E950



Ile Asp Lys Gln Leu Leu  
50

<210> 12

<211> 53

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
peptide

<400> 12

Gly Glu Pro Ile Ile Asn Phe Tyr Asp Pro Leu Val Phe Pro Ser Asp  
1 5 10 15

Glu Phe Asp Ala Ser Ile Ser Gln Val Asn Glu Lys Ile Asn Gln Ser  
20 25 30

Leu Ala Phe Ile Arg Lys Ser Asp Glu Leu Leu His Asn Val Asn Ala  
35 40 45

Gly Lys Ser Thr Thr  
50

<210> 13

<211> 48

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
peptide

<400> 13

Tyr Thr Ser Val Ile Thr Ile Glu Leu Ser Asn Ile Lys Glu Asn Lys  
1 5 10 15

Cys Asn Gly Thr Asp Ala Lys Val Lys Leu Ile Lys Gln Glu Leu Asp  
20 25 30

Lys Tyr Lys Asn Ala Val Thr Glu Leu Gln Leu Leu Met Gln Ser Thr  
35 40 45

<210> 14  
<211> 34  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic  
peptide

<400> 14  
Tyr Thr Ser Val Ile Thr Ile Glu Leu Ser Asn Ile Lys Glu Asn Lys  
1 5 10 15

Cys Asn Gly Asp Ala Lys Val Lys Leu Ile Lys Gln Glu Leu Asp Lys  
20 25 30

Tyr Lys

<210> 15  
<211> 34  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic  
peptide

<400> 15  
Thr Ser Val Ile Thr Ile Glu Leu Ser Asn Ile Lys Glu Asn Lys Cys  
1 5 10 15

Asn Gly Asp Ala Lys Val Lys Leu Ile Lys Gln Glu Leu Asp Lys Tyr  
20 25 30

Lys Asn

<210> 16  
<211> 34  
<212> PRT  
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
peptide

<400> 16

Val Ile Thr Ile Glu Leu Ser Asn Ile Lys Glu Asn Lys Cys Asn Gly  
1 5 10 15

Asp Ala Lys Val Lys Leu Ile Lys Gln Glu Leu Asp Lys Tyr Lys Asn  
20 25 30

Ala Val

<210> 17

<211> 34

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
peptide

<400> 17

Val Ile Thr Ile Glu Leu Ser Asn Ile Lys Glu Asn Lys Met Asn Gly  
1 5 10 15

Asp Ala Lys Val Lys Leu Ile Lys Gln Glu Leu Asp Lys Tyr Lys Asn  
20 25 30

Ala Val

<210> 18

<211> 33

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
peptide

<400> 18

Val Ala Val Ser Lys Val Leu His Leu Glu Gly Glu Val Asn Lys Ile  
1 5 10 15

Ala Leu Leu Ser Thr Asn Lys Ala Val Val Ser Leu Ser Asn Gly Val  
20 25 30

Ser

<210> 19

<211> 33

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
peptide

<400> 19

Ala Val Ser Lys Val Leu His Leu Glu Gly Glu Val Asn Lys Ile Ala  
1 5 10 15

Leu Leu Ser Thr Asn Lys Ala Val Val Ser Leu Ser Asn Gly Val Ser  
20 25 30

Val

<210> 20

<211> 33

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
peptide

<400> 20

Val Ser Lys Val Leu His Leu Glu Gly Glu Val Asn Lys Ile Ala Leu  
1 5 10 15

Leu Ser Thr Asn Lys Ala Val Val Ser Leu Ser Asn Gly Val Ser Val  
20 25 30

Leu

<210> 21

<211> 33  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic  
peptide

<400> 21  
Ser Lys Val Leu His Leu Glu Gly Glu Val Asn Lys Ile Ala Leu Leu  
1 5 10 15

Ser Thr Asn Lys Ala Val Val Ser Leu Ser Asn Gly Val Ser Val Leu  
20 25 30

Thr

<210> 22  
<211> 33  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic  
peptide

<400> 22  
Lys Val Leu His Leu Glu Gly Glu Val Asn Lys Ile Ala Leu Leu Ser  
1 5 10 15

Thr Asn Lys Ala Val Val Ser Leu Ser Asn Gly Val Ser Val Leu Thr  
20 25 30

Ser

<210> 23  
<211> 33  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic  
peptide

<400> 23

Leu Glu Gly Glu Val Asn Lys Ile Ala Leu Leu Ser Thr Asn Lys Ala  
1 5 10 15

Val Val Ser Leu Ser Asn Gly Val Ser Val Leu Thr Ser Lys Val Leu  
20 25 30

Asp

<210> 24

<211> 33

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
peptide

<400> 24

Gly Glu Val Asn Lys Ile Ala Leu Leu Ser Thr Asn Lys Ala Val Val  
1 5 10 15

Ser Leu Ser Asn Gly Val Ser Val Leu Thr Ser Lys Val Leu Asp Leu  
20 25 30

Lys

<210> 25

<211> 33

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
peptide

<400> 25

Glu Val Asn Lys Ile Ala Leu Leu Ser Thr Asn Lys Ala Val Val Ser  
1 5 10 15

Leu Ser Asn Gly Val Ser Val Leu Thr Ser Lys Val Leu Asp Leu Lys  
20 25 30

Asn

<210> 26  
<211> 33  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic  
peptide

<400> 26  
Val Asn Lys Ile Ala Leu Leu Ser Thr Asn Lys Ala Val Val Ser Leu  
1 5 10 15

Ser Asn Gly Val Ser Val Leu Thr Ser Lys Val Leu Asp Leu Lys Asn  
20 25 30

Tyr

<210> 27  
<211> 33  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic  
peptide

<400> 27  
Asn Lys Ile Ala Leu Leu Ser Thr Asn Lys Ala Val Val Ser Leu Ser  
1 5 10 15

Asn Gly Val Ser Val Leu Thr Ser Lys Val Leu Asp Leu Lys Asn Tyr  
20 25 30

Ile

<210> 28  
<211> 33  
<212> PRT  
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
peptide

<400> 28

Lys Ile Ala Leu Leu Ser Thr Asn Lys Ala Val Val Ser Leu Ser Asn  
1 5 10 15  
Gly Val Ser Val Leu Thr Ser Lys Val Leu Asp Leu Lys Asn Tyr Ile  
20 25 30

Asp

<210> 29

<211> 33

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
peptide

<400> 29

Ile Ala Leu Leu Ser Thr Asn Lys Ala Val Val Ser Leu Ser Asn Gly  
1 5 10 15  
Val Ser Val Leu Thr Ser Lys Val Leu Asp Leu Lys Asn Tyr Ile Asp  
20 25 30

Lys

<210> 30

<211> 33

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
peptide

<400> 30

Ala Leu Leu Ser Thr Asn Lys Ala Val Val Ser Leu Ser Asn Gly Val  
1 5 10 15





Ser Lys Glu Trp Ile Arg Arg Ser Asn Gln Lys Leu Asp Ser Ile Gly  
35 40 45

Asn Trp His Gln Ser Ser Thr Thr  
50 55

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<210> 33
<211> 35
<212> PRT
<213> Artificial Sequence
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<220>  
<223> Description of Artificial Sequence: synthetic peptide

<400> 33  
Thr Leu Asn Asn Ser Val Ala Leu Asp Pro Ile Asp Ile Ser Ile Glu  
1 5 10 15

Leu Asn Lys Ala Lys Ser Asp Leu Glu Glu Ser Lys Glu Trp Ile Arg  
20 25 30

Arg Ser Asn  
35

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<210> 34
<211> 35
<212> PRT
<213> Artificial Sequence
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<220>  
<223> Description of Artificial Sequence: synthetic peptide

<400> 34  
Leu Asn Asn Ser Val Ala Leu Asp Pro Ile Asp Ile Ser Ile Glu Leu  
1 5 10 15

Asn Lys Ala Lys Ser Asp Leu Glu Glu Ser Lys Glu Trp Ile Arg Arg  
20 25 30

Ser Asn Gln  
35

<210> 35  
<211> 35  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic  
peptide

<400> 35  
Asn Asn Ser Val Ala Leu Asp Pro Ile Asp Ile Ser Ile Glu Leu Asn  
1 5 10 15  
Lys Ala Lys Ser Asp Leu Glu Glu Ser Lys Glu Trp Ile Arg Arg Ser  
20 25 30  
Asn Gln Lys  
35

<210> 36  
<211> 35  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic  
peptide

<400> 36  
Asn Ser Val Ala Leu Asp Pro Ile Asp Ile Ser Ile Glu Leu Asn Lys  
1 5 10 15  
Ala Lys Ser Asp Leu Glu Glu Ser Lys Glu Trp Ile Arg Arg Ser Asn  
20 25 30  
Gln Lys Leu  
35

<210> 37  
<211> 35  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic  
peptide

<400> 37

Ser Val Ala Leu Asp Pro Ile Asp Ile Ser Ile Glu Leu Asn Lys Ala  
1 5 10 15

Lys Ser Asp Leu Glu Glu Ser Lys Glu Trp Ile Arg Arg Ser Asn Gln  
20 25 30

Lys Leu Asp  
35

<210> 38

<211> 35

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
peptide

<400> 38

Val Ala Leu Asp Pro Ile Asp Ile Ser Ile Glu Leu Asn Lys Ala Lys  
1 5 10 15

Ser Asp Leu Glu Glu Ser Lys Glu Trp Ile Arg Arg Ser Asn Gln Lys  
20 25 30

Leu Asp Ser  
35

<210> 39

<211> 35

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
peptide

<400> 39

Ala Leu Asp Pro Ile Asp Ile Ser Ile Glu Leu Asn Lys Ala Lys Ser  
1 5 10 15

Asp Leu Glu Glu Ser Lys Glu Trp Ile Arg Arg Ser Asn Gln Lys Leu  
20 25 30

Asp Ser Ile  
35

<210> 40  
<211> 35  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic  
peptide

<400> 40  
Leu Asp Pro Ile Asp Ile Ser Ile Glu Leu Asn Lys Ala Lys Ser Asp  
1 5 10 15

Leu Glu Glu Ser Lys Glu Trp Ile Arg Arg Ser Asn Gln Lys Leu Asp  
20 25 30

Ser Ile Gly  
35

<210> 41  
<211> 35  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic  
peptide

<400> 41  
Asp Pro Ile Asp Ile Ser Ile Glu Leu Asn Lys Ala Lys Ser Asp Leu  
1 5 10 15

Glu Glu Ser Lys Glu Trp Ile Arg Arg Ser Asn Gln Lys Leu Asp Ser  
20 25 30

Ile Gly Asn  
35

<210> 42  
<211> 35  
<212> PRT  
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
peptide

<400> 42

Pro Ile Asp Ile Ser Ile Glu Leu Asn Lys Ala Lys Ser Asp Leu Glu  
1 5 10 15

Glu Ser Lys Glu Trp Ile Arg Arg Ser Asn Gln Lys Leu Asp Ser Ile  
20 25 30

Gly Asn Trp  
35

<210> 43

<211> 35

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
peptide

<400> 43

Ile Asp Ile Ser Ile Glu Leu Asn Lys Ala Lys Ser Asp Leu Glu Glu  
1 5 10 15

Ser Lys Glu Trp Ile Arg Arg Ser Asn Gln Lys Leu Asp Ser Ile Gly  
20 25 30

Asn Trp His  
35

<210> 44

<211> 35

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
peptide

<400> 44

Asp Ile Ser Ile Glu Leu Asn Lys Ala Lys Ser Asp Leu Glu Glu Ser  
1 5 10 15

Lys Glu Trp Ile Arg Arg Ser Asn Gln Lys Leu Asp Ser Ile Gly Asn  
 20 25 30

Trp His Gln  
 35

<210> 45  
 <211> 35  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: synthetic  
 peptide

<400> 45  
 Ile Ser Ile Glu Leu Asn Lys Ala Lys Ser Asp Leu Glu Glu Ser Lys  
 1 5 10 15

Glu Trp Ile Arg Arg Ser Asn Gln Lys Leu Asp Ser Ile Gly Asn Trp  
 20 25 30

His Gln Ser  
 35

<210> 46  
 <211> 35  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: synthetic  
 peptide

<400> 46  
 Ser Ile Glu Leu Asn Lys Ala Lys Ser Asp Leu Glu Glu Ser Lys Glu  
 1 5 10 15

Trp Ile Arg Arg Ser Asn Gln Lys Leu Asp Ser Ile Gly Asn Trp His  
 20 25 30

Gln Ser Ser  
 35

<210> 47  
<211> 35  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic  
peptide

<400> 47  
Ile Glu Leu Asn Lys Ala Lys Ser Asp Leu Glu Glu Ser Lys Glu Trp  
1 5 10 15  
Ile Arg Arg Ser Asn Gln Lys Leu Asp Ser Ile Gly Asn Trp His Gln  
20 25 30  
Ser Ser Thr  
35

<210> 48  
<211> 35  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic  
peptide

<400> 48  
Glu Leu Asn Lys Ala Lys Ser Asp Leu Glu Glu Ser Lys Glu Trp Ile  
1 5 10 15  
Arg Arg Ser Asn Gln Lys Leu Asp Ser Ile Gly Asn Trp His Gln Ser  
20 25 30  
Ser Thr Thr  
35

<210> 49  
<211> 35  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic  
peptide



<400> 49

Thr Ala Ala Val Ala Leu Val Glu Ala Lys Gln Ala Arg Ser Asp Ile  
1 5 10 15

Glu Lys Leu Lys Glu Ala Ile Arg Asp Thr Asn Lys Ala Val Gln Ser  
20 25 30

Val Gln Ser  
35

<210> 50

<211> 35

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
peptide

<400> 50

Ala Val Ala Leu Val Glu Ala Lys Gln Ala Arg Ser Asp Ile Glu Lys  
1 5 10 15

Leu Lys Glu Ala Ile Arg Asp Thr Asn Lys Ala Val Gln Ser Val Gln  
20 25 30

Ser Ser Ile  
35

<210> 51

<211> 35

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
peptide

<400> 51

Leu Val Glu Ala Lys Gln Ala Arg Ser Asp Ile Glu Lys Leu Lys Glu  
1 5 10 15

Ala Ile Arg Asp Thr Asn Lys Ala Val Gln Ser Val Gln Ser Ser Ile  
20 25 30

Gly Asn Leu  
35

<210> 52  
<211> 35  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic  
peptide

<400> 52  
Val Glu Ala Lys Gln Ala Arg Ser Asp Ile Glu Lys Leu Lys Glu Ala  
1 5 10 15

Ile Arg Asp Thr Asn Lys Ala Val Gln Ser Val Gln Ser Ser Ile Gly  
20 25 30

Asn Leu Ile  
35

<210> 53  
<211> 35  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic  
peptide

<400> 53  
Glu Ala Lys Gln Ala Arg Ser Asp Ile Glu Lys Leu Lys Glu Ala Ile  
1 5 10 15

Arg Asp Thr Asn Lys Ala Val Gln Ser Val Gln Ser Ser Ile Gly Asn  
20 25 30

Leu Ile Val  
35

<210> 54  
<211> 35  
<212> PRT  
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
peptide

<400> 54

Ala Lys Gln Ala Arg Ser Asp Ile Glu Lys Leu Lys Glu Ala Ile Arg  
1 5 10 15

Asp Thr Asn Lys Ala Val Gln Ser Val Gln Ser Ser Ile Gly Asn Leu  
20 25 30

Ile Val Ala  
35

<210> 55

<211> 35

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
peptide

<400> 55

Lys Gln Ala Arg Ser Asp Ile Glu Lys Leu Lys Glu Ala Ile Arg Asp  
1 5 10 15

Thr Asn Lys Ala Val Gln Ser Val Gln Ser Ser Ile Gly Asn Leu Ile  
20 25 30

Val Ala Ile  
35

<210> 56

<211> 35

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
peptide

<400> 56

Gln Ala Arg Ser Asp Ile Glu Lys Leu Lys Glu Ala Ile Arg Asp Thr  
1 5 10 15



<210> 59  
<211> 35  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic  
peptide

<400> 59  
Ser Asp Ile Glu Lys Leu Lys Glu Ala Ile Arg Asp Thr Asn Lys Ala  
1 5 10 15  
Val Gln Ser Val Gln Ser Ser Ile Gly Asn Leu Ile Val Ala Ile Lys  
20 25 30  
Ser Val Gln  
35

<210> 60  
<211> 35  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic  
peptide

<400> 60  
Lys Leu Lys Glu Ala Ile Arg Asp Thr Asn Lys Ala Val Gln Ser Val  
1 5 10 15  
Gln Ser Ser Ile Gly Asn Leu Ile Val Ala Ile Lys Ser Val Gln Asp  
20 25 30  
Tyr Val Asn  
35

<210> 61  
<211> 35  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic  
peptide

<400> 61

Leu Lys Glu Ala Ile Arg Asp Thr Asn Lys Ala Val Gln Ser Val Gln  
1 5 10 15

Ser Ser Ile Gly Asn Leu Ile Val Ala Ile Lys Ser Val Gln Asp Tyr  
20 25 30

Val Asn Lys  
35

<210> 62

<211> 35

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
peptide

<400> 62

Ala Ile Arg Asp Thr Asn Lys Ala Val Gln Ser Val Gln Ser Ser Ile  
1 5 10 15

Gly Asn Leu Ile Val Ala Ile Lys Ser Val Gln Asp Tyr Val Asn Lys  
20 25 30

Glu Ile Val  
35

<210> 63

<211> 47

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
peptide

<400> 63

Thr Trp Gln Glu Trp Glu Arg Lys Val Asp Phe Leu Glu Glu Asn Ile  
1 5 10 15

Thr Ala Leu Leu Glu Glu Ala Gln Ile Gln Gln Glu Lys Asn Met Tyr  
20 25 30

Glu Leu Gln Lys Leu Asn Ser Trp Asp Val Phe Gly Asn Trp Phe  
 35 40 45

<210> 64  
 <211> 35  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: synthetic  
 peptide

<400> 64  
 Trp Gln Glu Trp Glu Arg Lys Val Asp Phe Leu Glu Glu Asn Ile Thr  
 1 5 10 15

Ala Leu Leu Glu Glu Ala Gln Ile Gln Gln Glu Lys Asn Met Tyr Glu  
 20 25 30

Leu Gln Lys  
 35

<210> 65  
 <211> 35  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: synthetic  
 peptide

<400> 65  
 Gln Glu Trp Glu Arg Lys Val Asp Phe Leu Glu Glu Asn Ile Thr Ala  
 1 5 10 15

Leu Leu Glu Glu Ala Gln Ile Gln Gln Glu Lys Asn Met Tyr Glu Leu  
 20 25 30

Gln Lys Leu  
 35

<210> 66  
 <211> 35  
 <212> PRT  
 <213> Artificial Sequence

005060"EESEEE60

<220>

<223> Description of Artificial Sequence: synthetic  
peptide

<400> 66

Glu Trp Glu Arg Lys Val Asp Phe Leu Glu Glu Asn Ile Thr Ala Leu  
1 5 10 15

Leu Glu Glu Ala Gln Ile Gln Gln Glu Lys Asn Met Tyr Glu Leu Gln  
20 25 30

Lys Leu Asn  
35

<210> 67

<211> 35

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
peptide

<400> 67

Trp Glu Arg Lys Val Asp Phe Leu Glu Glu Asn Ile Thr Ala Leu Leu  
1 5 10 15

Glu Glu Ala Gln Ile Gln Gln Glu Lys Asn Met Tyr Glu Leu Gln Lys  
20 25 30

Leu Asn Ser  
35

<210> 68

<211> 35

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
peptide

<400> 68

Glu Arg Lys Val Asp Phe Leu Glu Glu Asn Ile Thr Ala Leu Leu Glu  
1 5 10 15



Glu Ala Gln Ile Gln Gln Glu Lys Asn Met Tyr Glu Leu Gln Lys Leu  
 20 25 30

Asn Ser Trp  
 35

<210> 69  
 <211> 35  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: synthetic  
 peptide

<400> 69  
 Arg Lys Val Asp Phe Leu Glu Glu Asn Ile Thr Ala Leu Leu Glu Glu  
 1 5 10 15

Ala Gln Ile Gln Gln Glu Lys Asn Met Tyr Glu Leu Gln Lys Leu Asn  
 20 25 30

Ser Trp Asp  
 35

<210> 70  
 <211> 35  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: synthetic  
 peptide

<400> 70  
 Lys Val Asp Phe Leu Glu Glu Asn Ile Thr Ala Leu Leu Glu Glu Ala  
 1 5 10 15

Gln Ile Gln Gln Glu Lys Asn Met Tyr Glu Leu Gln Lys Leu Asn Ser  
 20 25 30

Trp Asp Val  
 35

<210> 71  
<211> 35  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic  
peptide

<400> 71  
Val Asp Phe Leu Glu Glu Asn Ile Thr Ala Leu Leu Glu Glu Ala Gln  
1 5 10 15  
Ile Gln Gln Glu Lys Asn Met Tyr Glu Leu Gln Lys Leu Asn Ser Trp  
20 25 30  
Asp Val Phe  
35

<210> 72  
<211> 35  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic  
peptide

<400> 72  
Asp Phe Leu Glu Glu Asn Ile Thr Ala Leu Leu Glu Glu Ala Gln Ile  
1 5 10 15  
Gln Gln Glu Lys Asn Met Tyr Glu Leu Gln Lys Leu Asn Ser Trp Asp  
20 25 30  
Val Phe Gly  
35

<210> 73  
<211> 35  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic  
peptide

<400> 73

Phe Leu Glu Glu Asn Ile Thr Ala Leu Leu Glu Glu Ala Gln Ile Gln  
1 5 10 15

Gln Glu Lys Asn Met Tyr Glu Leu Gln Lys Leu Asn Ser Trp Asp Val  
20 25 30

Phe Gly Asn  
35

<210> 74

<211> 35

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
peptide

<400> 74

Pro Asp Ala Val Tyr Leu His Arg Ile Asp Leu Gly Pro Pro Ile Ser  
1 5 10 15

Leu Glu Arg Leu Asp Val Gly Thr Asn Leu Gly Asn Ala Ile Ala Lys  
20 25 30

Leu Glu Asp  
35

<210> 75

<211> 34

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
peptide

<400> 75

Leu Glu Arg Leu Asp Val Gly Thr Asn Leu Gly Asn Ala Ile Ala Lys  
1 5 10 15

Leu Glu Ala Lys Glu Leu Leu Glu Ser Ser Asp Gln Ile Leu Arg Ser  
20 25 30

Met Lys

<210> 76

<211> 34

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
peptide

<400> 76

Leu His Arg Ile Asp Leu Gly Pro Pro Ile Ser Leu Glu Arg Leu Asp  
1 5 10 15

Val Gly Thr Asn Leu Gly Asn Ala Ile Ala Lys Leu Glu Ala Lys Glu  
20 25 30

Leu Leu

<210> 77

<211> 34

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
peptide

<400> 77

His Arg Ile Asp Leu Gly Pro Pro Ile Ser Leu Glu Arg Leu Asp Val  
1 5 10 15

Gly Thr Asn Leu Gly Asn Ala Ile Ala Lys Leu Glu Ala Lys Glu Leu  
20 25 30

Leu Glu

<210> 78

<211> 34

<212> PRT

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peptide

<400> 78

Arg Ile Asp Leu Gly Pro Pro Ile Ser Leu Glu Arg Leu Asp Val Gly  
1 5 10 15

Thr Asn Leu Gly Asn Ala Ile Ala Lys Leu Glu Ala Lys Glu Leu Leu  
20 25 30

Glu Ser

<210> 79

<211> 34

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
peptide

<400> 79

Ile Asp Leu Gly Pro Pro Ile Ser Leu Glu Arg Leu Asp Val Gly Thr  
1 5 10 15

Asn Leu Gly Asn Ala Ile Ala Lys Leu Glu Ala Lys Glu Leu Leu Glu  
20 25 30

Ser Ser

<210> 80

<211> 34

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
peptide

<400> 80

Asp Leu Gly Pro Pro Ile Ser Leu Glu Arg Leu Asp Val Gly Thr Asn  
1 5 10 15

Leu Gly Asn Ala Ile Ala Lys Leu Glu Ala Lys Glu Leu Leu Glu Ser  
 20 25 30

Ser Asp

<210> 81  
 <211> 34  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: synthetic  
 peptide

<400> 81  
 Leu Gly Pro Pro Ile Ser Leu Glu Arg Leu Asp Val Gly Thr Asn Leu  
 1 5 10 15

Gly Asn Ala Ile Ala Lys Leu Glu Ala Lys Glu Leu Leu Glu Ser Ser  
 20 25 30

Asp Gln

<210> 82  
 <211> 34  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: synthetic  
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<400> 82  
 Gly Pro Pro Ile Ser Leu Glu Arg Leu Asp Val Gly Thr Asn Leu Gly  
 1 5 10 15

Asn Ala Ile Ala Lys Leu Glu Ala Lys Glu Leu Leu Glu Ser Ser Asp  
 20 25 30

Gln Ile

<210> 83  
<211> 34  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic  
peptide

<400> 83  
Pro Pro Ile Ser Leu Glu Arg Leu Asp Val Gly Thr Asn Leu Gly Asn  
1 5 10 15

Ala Ile Ala Lys Leu Glu Ala Lys Glu Leu Leu Glu Ser Ser Asp Gln  
20 25 30

Ile Leu

<210> 84  
<211> 34  
<212> PRT  
<213> Artificial Sequence

<220>  
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peptide

<400> 84  
Pro Ile Ser Leu Glu Arg Leu Asp Val Gly Thr Asn Leu Gly Asn Ala  
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Ile Ala Lys Leu Glu Ala Lys Glu Leu Leu Glu Ser Ser Asp Gln Ile  
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Leu Arg

<210> 85  
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peptide

<400> 85

Ser Leu Glu Arg Leu Asp Val Gly Thr Asn Leu Gly Asn Ala Ile Ala  
1 5 10 15

Lys Leu Glu Ala Lys Glu Leu Leu Glu Ser Ser Asp Gln Ile Leu Arg  
20 25 30

Ser Met

<210> 86

<211> 34

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
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<400> 86

Leu Glu Arg Leu Asp Val Gly Thr Asn Leu Gly Asn Ala Ile Ala Lys  
1 5 10 15

Leu Glu Ala Lys Glu Leu Leu Glu Ser Ser Asp Gln Ile Leu Arg Ser  
20 25 30

Met Lys

U06060-453330



Atty Docket No. REDC-1510 USA

**COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY**

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

LONG LASTING FUSION PEPTIDE INHIBITORS OF VIRAL INFECTION

the specification of which (check one) ☐ is attached hereto or ☒ was filed on 17 May 2000 as International Application No. PCT/US00/13651 and was amended on \_\_\_\_\_ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information which is material to patentability as defined in 37 CFR § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)			Priority Claimed	
			Yes	No
Number	Country	Day/Month/Year Filed		
Number	Country	Day/Month/Year Filed		

I hereby claim the benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) below.

<u>60/134,406</u>	<u>17 May 1999</u>
Application Number	Filing Date
<u>60/153,406</u>	<u>10 September 1999</u>
Application Number	Filing Date

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose all information which is material to patentability as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

Application Number	Filing Date	Status: Patented, Pending, Abandoned
Application Number	Filing Date	Status: Patented, Pending, Abandoned

I HEREBY APPOINT THE FOLLOWING AS MY ATTORNEYS WITH FULL POWER OF SUBSTITUTION TO PROSECUTE THIS APPLICATION AND TRANSACT ALL BUSINESS IN THE PATENT OFFICE CONNECTED THEREWITH:

Karl A. Limbach	18,689	Alfred A. Equitz	30,922	Mayumi Maeda	40,075
George C. Limbach	19,305	Charles P. Sammut	28,901	Michael R. Ward	38,651
John K. Uilkema	20,282	Mark C. Pickering	36,239	Roger S. Sampson	44,314
Neil A. Smith	25,441	Patricia Coleman James	37,155	Charles L. Hamilton	42,624
Veronica C. Devitt	29,375	Kathleen A. Frost	37,326	Andrew V. Smith	43,132
Ronald L. Yin	27,607	Alan A. Limbach	39,749	Eric N. Hoover	37,355
Gerald T. Sekimura	30,103	Douglas C. Limbach	35,249	Frank J. Mycroft	P-46,946
Michael A. Stallman	29,444	Seong-Kun Oh*		Parisa Jorjani	P-46,813
Philip A. Girard	28,848	Cameron A. King	41,897	Robert M. McConnell	P-46,912
Michael J. Pollock	29,098	Kyla L. Harriel	41,816	J. Thomas McCarthy	22,420
Stephen M. Everett	30,050			Joel G. Ackerman	24,307

\* Recognition under 37 CFR 10.9(b)

Send correspondence to Limbach & Limbach L.L.P.  
Attn: Michael R. Ward, Esq.  
2001 Ferry Building  
San Francisco, CA 94111  
Telephone: 415/433-4150

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under 18 U.S.C. § 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first inventor DOMINIQUE P. BRIDON

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 Citizenship France  
 Post Office Address 243 Chemin Cote Ste-Catherine, Outremont, Quebec H2V 2B2, Canada

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Inventor's signature \_\_\_\_\_ Date \_\_\_\_\_  
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 Citizenship United States of America  
 Post Office Address 115 Pilgrim Road, Wellseley, Massachusetts 02181

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Inventor's signature \_\_\_\_\_ Date \_\_\_\_\_  
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 Citizenship Canada, Algeria  
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Inventor's signature \_\_\_\_\_

Date \_\_\_\_\_

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Citizenship Canada

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Full name of fifth joint inventor, if any, PETER G. MILNER

Inventor's signature Peter G. Milnes 8/22/2000

Date \_\_\_\_\_

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Citizenship United Kingdom

Post Office Address 14690 Manuella Road, Los Altos Hills, California 94022[illegible]

Atty Docket No. REDC-1510 USA

**COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY**

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**LONG LASTING FUSION PEPTIDE INHIBITORS OF VIRAL INFECTION**

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			Yes	No
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Application Number	Filing Date	Status: Patented, Pending, Abandoned
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Ronald L. Yin	27,607	Alan A. Limbach	39,749	Eric N. Hoover	37,355
Gerald T. Sekimura	30,103	Douglas C. Limbach	35,249	Frank J. Mycroft	P-46,946
Michael A. Stallman	29,444	Seong-Kun Oh*		Parisa Jorjani	P-46,813
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Michael J. Pollock	29,098	Kyla L. Harriel	41,816	J. Thomas McCarthy	22,420
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Full name of sole or first inventor DOMINIQUE P. BRIDON

Inventor's signature [Signature]

15 August 2000

PQC Date

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Full name of second joint inventor, if any, ROBERT X DUFRESNE

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Aug 15, 2000

Date

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Citizenship United States of America

Post Office Address 115 Pilgrim Road, Wellseley, Massachusetts 02181

Full name of third joint inventor, if any, NISSAB BOUDJELLAB

Inventor's signature [Signature]

Date

Aug 15, 2000

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Citizenship Canada, Algeria

Post Office Address 420 Bourke, Apt. 2A, Dorval, Quebec H9A 2M1, Canada

H9S 3X1

Full name of fourth joint inventor, if any, MARTIN ROBITAILLE

Inventor's signature Lab. T. M. 15 Aug 2000

Date \_\_\_\_\_

Residence 108 2 ieme Boulevard, Terrasse-Vaudreuil, Quebec J7V 5S1, Canada

Citizenship Canada

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Full name of fifth joint inventor, if any, PETER G. MILNER

Inventor's signature \_\_\_\_\_

Date \_\_\_\_\_

Residence 14690 Manuella Road, Los Altos Hills, California 94022

Citizenship United Kingdom

Post Office Address 14690 Manuella Road, Los Altos Hills, California 94022